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BIOLOGICAL FEEDBACK

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Biological Feedback

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R. Thomas's main discoveries are the hyperchromic effect and denaturation of nucleic acids (1951), the direct translational control of replication ("Thomas-Bertani" effect, 1964), a clear demonstration of the experimental occurrence of positive controls (1965), and a new vision of the biological role of positive and negative feedback loops. He developed a powerful logical tool ("kinetic logic") which, in its generalized form, is especially appropriate for the treatment of systems comprising multiple feedback loops.

René Thomas is a member of the Académie Royale de Belgique (1975), the New York Academy of Sciences (1963), and the Academia Europaea (1989). He was the laureate of the most prestigious Belgian scientific prizes, the Francqui Prize (1975) and the Prix Quinquennal du Fonds National de la Recherche Scientifique (1986).

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PROLOGUE: PHILOSOPHY OF THE BOOK

I. REGULATION

Regulation is one of the most fascinating aspects of biology. The term "biological regulation" covers such diverse phenomena as temperature control in warm-blooded animals; differentiation of a zygote into the various specialized organs, tissues, and cells of the mature organism; the fate of certain temperate bacteriophage, which after infection can either produce more phage and kill their host or integrate into the host genome and repress expression of phage genes. Ultimately, we expect most, if not all, biological regulation to be understandable at the level of gene expression.

Regulation may be defined as the constraints that adjust the rate of production of the elements of a system to the state of the system and of relevant environmental variables. We are particularly interested in specific, rather than general, regulation. If, for example, a gene product specifically affects the rate of expression of another gene (or a small set of genes), we consider the interaction regulatory, unlike, say, the increased rate of expression of all genes observed in cold-blooded organisms when the temperature rises.

II. HOMEOSTATIC VS. EPIGENETIC REGULATION

When thinking about regulation — biological or other — people generally have in mind *homeostatic* regulation, that is, regulation that maintains the level of a variable at or near a fixed (supposedly optimal) value. The classic example of a homeostatic regulator is the thermostat, of which many natural and man-made variants exist. When the temperature reaches a particular value, heat *production* stops, and when it falls below this value, heat production starts again. The temperature is thus adjusted by switching heat production on and off, i.e., by alternating between upward and downward corrections. Another example of homeostatic regulation is the well-known phenomenon of end-product inhibition, whereby the final product of a biosynthetic pathway inhibits the first reaction of the pathway; this tends to maintain a constant pool of the metabolite.

It is perhaps worth pointing out that this regulation not only *maintains* a variable at a fixed level, but also *adjusts* the variable to that level, even if the initial value is quite different. A thermostat will adjust the temperature to the same steady-state value regardless of what the initial temperature of the system may be, and metabolite pools will be brought to the same level whatever their initial size. Provided the initial conditions are not beyond the range in which the regulation is operative, the homeostatic system will evolve toward the same final state.

In practice, even though a thermostat (or end-product inhibition) tends to maintain a constant temperature (or metabolite pool), the control exerted may result in an oscillation around the desired value. More generally, homeostatic regulation tends to maintain a variable at or near a fixed value which is intermediate between those that would prevail if the producer were permanently *on* or permanently *off*. The oscillation (if any) around an average value may, according to the case, be merely an imperfection of the stabilizing device or a fundamental aspect of the regulation.

A Leitmotiv of this book is that one should attach at least equal importance to another type of regulation, referred to here as *epigenetic* or differentiative. Instead of fixing a variable at an intermediate level, it provides a choice between extreme levels. Once the choice is made, in the absence of environmental change, the system will remain indefinitely in the chosen state. A simple example of this type of regulation is the "safety" gas pilot light,

designed to stop the gas flow if the flame goes out. The gas flows through a special valve that is placed above the flame and is open only when heated. The system can be either *on* — flame lit, valve heated, gas flowing — or *off* — no flame, valve closed, no gas flow. Any autocatalytic process will exhibit this type of regulation. A biological example would be a protein required for its own synthesis (many such are known). Again, there are two stable states: the protein can be present (and actively synthesized) or absent (and not synthesized). Temporary inactivation of the protein, e.g., by thermal denaturation during a heat shock, will make it impossible to resume synthesis, even after the temperature has returned to its normal value; the system is stably “off”.

The salient feature of this type of regulation is that a *transient* change in the environment — e.g., temporary extinction of the flame or heat shock — can result in a *stable* change of state of the system, continuing long after the perturbation. A second aspect of the regulation is that it resembles a vicious circle: the system is unable to switch on spontaneously, but once on, it maintains itself.

In general, systems of this type can stabilize in several different ways. There can be specific points (stable steady states) toward which the system tends to move. Alternatively, the system can oscillate permanently around a steady state (stable cycles), or it can exhibit more complex (aperiodic or chaotic) behavior. In all cases, the final state or path is called an *attractor*. The number of attractors of such a system need not be limited to two; examples will be encountered with many stable states. One of our considerations in this book will be to evaluate the number of attractors in a given system and to determine toward which final situation a given initial state will evolve. Why this type of regulation is called “epigenetic” will appear in a later chapter. Its importance stems from the fact that it is probably a major mechanism in cell differentiation. This possibility was already mentioned by Delbrück¹ in an epoch-making remark, whose translation from French can be found in Chapter 17, Section I. He states, “The above proposition is not new, and many biologists have a fairly clear idea of what it implies.” Ironically, most of us would be hard put to enumerate these “many biologists” — except perhaps Waddington. We finally, in a provocative review by Rosen², found references not only to Waddington,³ but also to Nanney,⁴ and Needham;⁵ however, none is anterior to Delbrück. On the other hand, models generating multiple steady states (of which epigenetic differences are clearly a special case) had been developed earlier by Rashevsky⁶ and by Turing.⁷ The fundamental basis of this multistationarity is now understood in terms of the thermodynamics of open systems (Glansdorff and Prigogine⁸, Nicolis and Prigogine⁹).

III. BIOLOGY: A HARD OR SOFT SCIENCE?

Biology, because of its complexity, has traditionally been a soft science. Has the fulgurant progress in genetics and biochemistry made it harder? Our present detailed knowledge of *individual* molecular mechanisms involved in biological reactions can certainly be considered hard science. Indeed, there are many cases of gene regulation, for example, that are understood in precise molecular detail. However, the *global* operation of biological systems has remained a soft science. Symptomatic of this is the fact that networks of interactions are still described in verbal terms or as cartoons rather than being formalized.

IV. LEVELS OF DESCRIPTION: VERBAL, LOGICAL, DIFFERENTIAL

The quantitative mathematical description of a biological system generally involves systems of differential equations. These implicitly contain the complete kinetic behavior of the system, i.e., its state as a function of time, starting from any initial state; we call it a differential description. The differential equations involved in biological regulation are often

highly nonlinear, and even simple cases cannot be solved analytically; more complex systems almost inevitably lead one to oversimplify the models.

Until recently there was no intermediate level of description between the purely *verbal* and the *differential* descriptions. One particularity of our approach is the introduction and wide use of a *logical* (or Boolean) description, which fills this gap (see references in Chapter 1). Logical analysis uses discontinuous variables and functions with a limited number of values, often only two. Basically, to each variable we try to attribute as few distinct values as there are *qualitatively* distinct levels. This emphasizes the essential qualitative features of the system at the expense of kinetic and mechanistic details. For example, if the biological role of a certain protein is to turn on a specific gene, we may consider that the protein is either present or absent, with the gene respectively on or off. Similarly, in the case of a thermostat, the temperature can be essentially characterized as being above, within, or below the narrow range toward which the system evolves.

In this book, we will use all three levels of description — verbal, logical, and differential — often in parallel. Our goals in formalizing the description of biological systems will be to determine all the attractors of a system and, when there is more than one, to determine the qualitative constraints on the parameter values that will direct the system from a given initial state toward one or another attractor. As we shall see, the logical description is particularly suited to this analysis, with simple algorithms for extracting the desired information. The differential description can provide the detailed kinetic evolution of the system, using numerical approximations with parameter values suggested by the logical analysis.

V. REGULATORY FUNCTIONS, SIGMOID CURVES, AND FEEDBACK LOOPS

Our analysis considers systems whose elements can interact positively or negatively, that is, the *level* of an element may activate or reduce the *rate of production* of other elements or of itself. If a product a acts to stimulate the synthesis of b , it is a positive regulator. In such situations, the rate of synthesis of b increases with increasing concentration of a , generally following a curve similar to that shown in Figure 1A. It can be seen that there is little effect of a until it reaches a threshold concentration θ , and at higher concentrations a plateau is reached, representing the maximal rate of synthesis of b . Such a nonlinear, bounded curve, called a *sigmoid*, is typical of regulatory interactions in biology. It suggests that it is justifiable to reason as though a were “absent” for $a < \theta$ and “present” for $a > \theta$; in other words, we approximate the sigmoid curve of Figure 1A by the *step function* of Figure 1B.

When elements are connected in a topologically circular way, thus exerting an influence on their own rate of production, they form a *feedback loop*. Those familiar with graph theory, in which the term “loop” refers to a specific structure, may object to our using it in such a general way. However, “feedback loop” is such a familiar expression in modern biology that we have preferred to keep it. Throughout this book, “loop” will be used only in the general sense defined here. A loop containing n elements will thus involve n interactions, each of which can be positive or negative. As we shall see, the two types of regulation mentioned above are based on two types of feedback loop. “Interesting” systems are often networks comprising several intertwined feedback loops. Much of our work, partly described in this book, has consisted of establishing algorithms for treating complex systems involving several interconnected feedback loops.

VI. LOGICAL VS. STRUCTURAL COMPLEXITIES

It may be worth pointing out here that descriptions of similar *logical complexity* may apply to systems having very different degrees of *structural complexity*. The elements of the

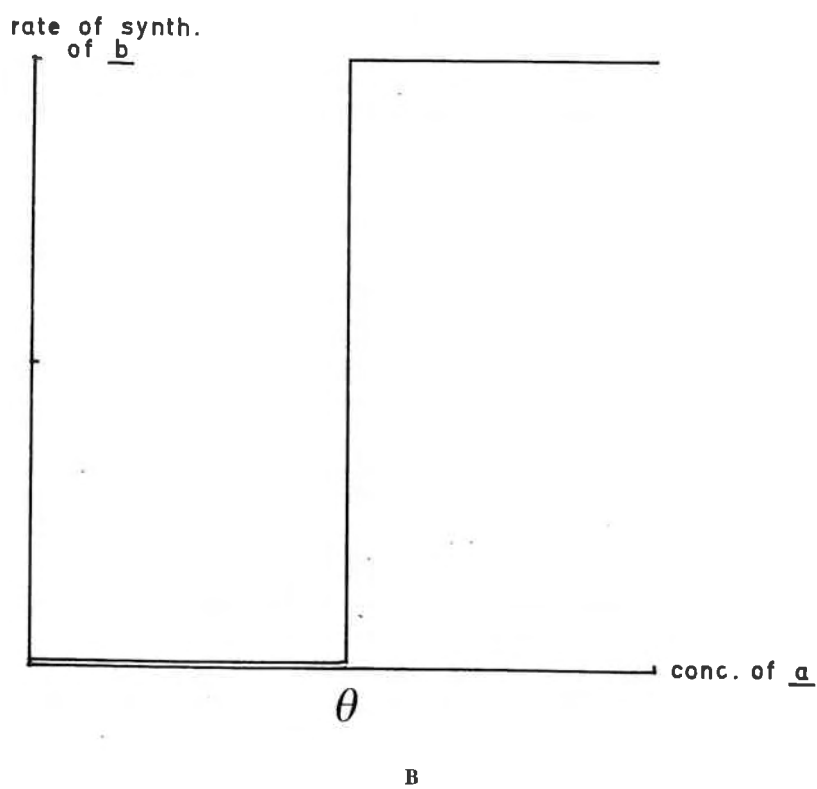
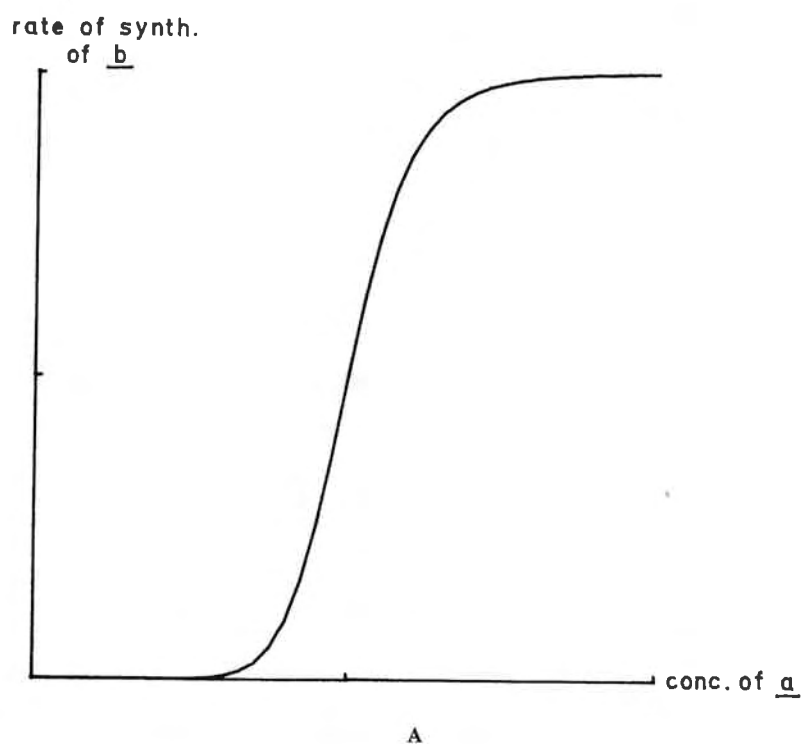


FIGURE 1. (A) Sigmoid relation between the concentration of the regulator a and the rate of synthesis of b . (B) Approximation by a step function.

systems we analyze may be molecules, organelles, cells, organs, or organisms, if not societies and, accordingly, the physical nature of the interactions between elements will differ widely from case to case. Nevertheless, if two systems have a similar logical structure, the essential qualitative features of their dynamics will be similar. This is why the book will deal with systems as different as bacteriophage, sets of neurons, and the immune response. However, as geneticists, we will speak mostly in terms of gene expression.

Genes are segments of DNA, part of whose nucleotide sequence codes for a specific product, most generally a particular protein. Gene expression takes place in several steps. First, the DNA sequence serves as a template for the synthesis of a similar sequence of "messenger RNA" (mRNA). This process is called "transcription" because the template and the mRNA use the same alphabet of four nucleotides. The mRNA molecule may undergo certain chemical modifications and splicing. It then serves as a model for the synthesis of a specific protein, consisting of a sequence of amino acids, of which 20 different types are used. This process is called "translation" because it involves a change from one language to another. The protein itself may then be modified chemically before forming the final active gene product. Most of the time, "gene expression" will be used here in a broad acceptance, referring to the entire chain of events from transcription to formation of an active product. This is sufficient for the frequently encountered situation in which regulation is exerted at the transcriptional level: such a gene is "on" if it is being actively transcribed into mRNA, "off" otherwise. If regulation is exerted at the level of mRNA modification, splicing, translation, or posttranslational events, these features can be included in a more detailed description with no particular difficulties.

VII. WHAT CAN BE DESCRIBED LOGICALLY?

Roughly speaking, logical formalism can give a rigorous description of a system which is by its nature discontinuous, or an idealized description of a system which is fundamentally continuous. Insofar as most systems are continuous at the scale we use, the logical description is often felt to be a crude simplification. In fact, this view is itself an oversimplification. Both logical and differential descriptions involve simplifying assumptions, which will be discussed in later chapters. Here, we will just mention several relevant points.

Unless a system has been subjected to a fully quantitative mathematical treatment (in which the underlying logical relations are built into the equations), formal logic is routinely used only in small, disjoint patches in the description of a biological system; sections whose logical complexity does not exceed that of a simple syllogism are extricated from complete networks and treated separately. In fact, if a network (or model) has been formulated verbally, it can be formalized in its entirety, and the information extracted from such a global description is vastly richer and, furthermore, obtained by the use of simple algorithms; these procedures are described in Chapter 3. We also provide straightforward tools for generalizing the logical description to cover situations of considerably greater logical complexity than the syllogism (cf. Chapter 2).

Not surprisingly, one problem has been to construct a logic in which *time* is included in a logically acceptable, yet convenient, manner. Our logical description handles this problem by the use of time delays for all relevant processes (e.g., the time required for a gene product to reach its threshold concentration after the gene has been turned on). The number of time delays used and their actual numerical values do not complicate the formal logical description, and it is a simple matter to derive qualitative relations among the time delays which determine the qualitative dynamic behavior of the system, i.e., the sequence of states followed from a given initial state to one or another attractor (final stable cycle or state); these procedures are described in Chapter 4. Curiously, the traditional differential description, although it intrinsically contains the complete kinetic behavior of the system, is consider-

ably less supple as regards time delays, the introduction of which constitutes a major mathematical complication. This point is discussed in detail in Chapter 4.

As we shall see, our logical description extracts the qualitatively essential features of the dynamics of a system and provides an image which is, *mutatis mutandis*, similar to that given by differential analysis; the picture is more schematic, but there is little loss of *qualitative* resolution.

VIII. HOW TO USE THIS BOOK

Chapter 6, which deals with continuous descriptions, involves a certain amount of detail about systems of differential equations. People allergic to such treatments can skip Chapters 6, 10, and 12 without missing the basic message. Similarly, those who do not care to mar logical elegance with the crude realities of actual biological systems can skip Part 3. Elimination of all these sections would transform the work into a textbook on the formal logical treatment of systems comprising feedback loops.

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Part I: Tools



Chapter 1

INTRODUCTION TO PART I

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I. LOGICAL VS. DIFFERENTIAL DESCRIPTIONS

The biological systems we are interested in are almost always described verbally or by cartoons. When they are formalized, it is usually by sets of ordinary differential equations which give the time derivative of each relevant element as a function of the state of the system (concentration of the elements, temperature, etc.). We describe the differential formalization in Chapter 6.

In addition, as already alluded to in the Prologue, we make extensive use of a "logical" (or "Boolean") description. This description uses variables and functions with a limited number of values — in simple cases, only two: 0 and 1. To begin with, we associate logical variables with the relevant elements of the system and describe the *state* of the system by a *logical vector*, which consists of the logical values of these variables presented in a defined order. Consider, for example, a system whose state is appropriately described by the levels of substances *a*, *b*, and *c*, each of which can be absent, present at low level, or present at high level (logical values 0, 1, and 2, respectively). The situation in which *a* is absent, *b* is present at high level, and *c* is present at low level will be described by the logical vector **021**.

A number of authors before us or more recently have proposed logical descriptions of biological processes and established important properties of systems comprising feedback loops (Rashevsky¹, Sugita², Kauffman³, Glass¹³, Wolpert and Lewis¹⁴). Despite superficial similarities, our description⁴⁻⁸ differs considerably from previous ones. Our method, called *kinetic logic*, will be outlined in Section IV of this chapter and described at length in Chapters 3, 4, 5, and 7. First, however, we must briefly introduce some fundamental notions of logic (Chapter 2).

II. COMBINATORIAL VS. SEQUENTIAL LOGICAL CIRCUITS

A. COMBINATORIAL LOGICAL CIRCUITS

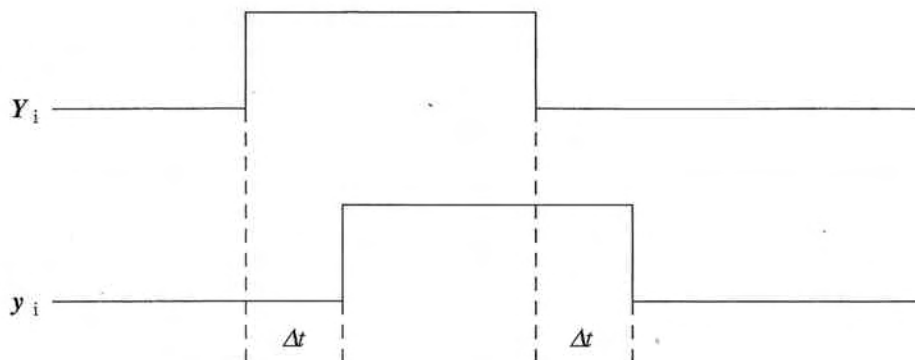
Combinatorial logical circuits can be compared to electrical circuits with switches and lamps wired so that each lamp will be on or off, depending only on which switches are on or off; the state (on or off) of each lamp is a function of the *present* state (on or off) of the set of switches. The switches are the *variables* of the system and the lamps are *functions* of these variables. More precisely, we call the switches *input variables* because their values can be decided arbitrarily and the lamps *output functions* because they do not exert any retroaction (feedback) on the state of the system. Combinatorial systems will be dealt with at length in Chapter 2. However, this type of simple circuit is inadequate for describing biological systems, in part because it does not include time and because in biology, feedback, both positive and negative, is the heart of regulation.

B. SEQUENTIAL LOGICAL CIRCUITS

In *sequential logical circuits*, there are functions whose value depend not only on the *present* values of the input variables, but also on *former* values of these variables and on the order in which they have changed. Consider, for example, a system with a button (variable *x*) and a lamp (function *X*) which can be lit by pushing the button (*x* = 1), but which will then remain on even after the button is released (*x* = 0). When *x* = 1, the lamp is always on (*X* = 1), but when *x* = 0, the lamp may be on or off, depending on whether the variable *x* has had the value 1 sometime in the past, or, more simply, whether the function *X* had the value 1 in the immediate past.

In this field, a fundamental, indeed ingenious, step forward consisted in the introduction of so-called *internal functions* and *variables*, which formally permit one to convert a sequential problem into a combinatorial one (Huffman⁹, Florine¹⁰). Here, we will simply state that

internal functions and variables, as used in sequential logic, are formal entities related as follows: an internal function Y_i and its associated internal variable y_i have the same logical value in steady-state (unchanging) conditions, and when the value of the function changes, the value of the variable follows after a short time Δt :



The value of the variable thus serves as a memory of the preceding value of its associated function. In this way, instead of relating a function to a *previous* value of the function Y_i , one can relate it to the *present* value of the associated variable y_i . Thus, for appropriately chosen internal functions and variables, any function X can be expressed in terms of the *present* values of the input variables and internal variables. This, in short, is how sequential systems are formally converted into combinatorial systems. As will be seen below, this type of circuit, as such, is still inadequate for describing biological systems realistically.

III. INTRODUCTION OF TIME: SYNCHRONOUS VS. ASYNCHRONOUS DESCRIPTION

To deal with dynamic systems, and in particular biological systems, *time* must be introduced into the logical formalism. The problem was to introduce it in a conceptually acceptable way and yet keep the formalism simple enough to remain attractive and workable for nonmathematicians.

The classical approach relates the system at time $t + 1$ to its state at time t . More is said about this type of description in Appendix 4. Here, we are concerned with its usefulness for describing biological systems and will therefore only mention the following relevant points:

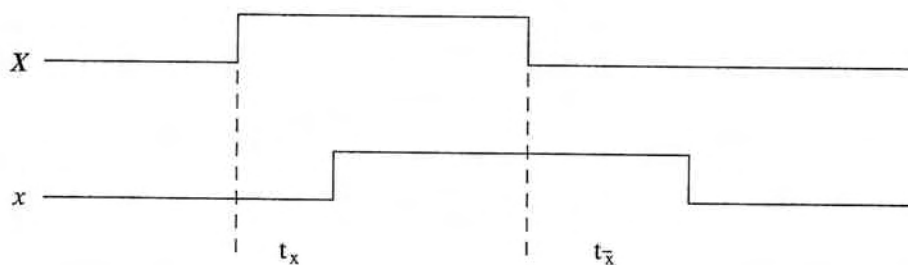
1. In this description, each logical state has one and only one possible successor. Thus, from any initial state, the system will follow a well-defined pathway, without any branching or possibility of choice (see Appendix 4, Figure 1).
2. When $(abcd)_{t+1}$ differs from $(abcd)_t$ by the values of more than one variable, the logical values of all these variables change simultaneously, exactly when the clock reaches $t + 1$. For this reason, the description is called *synchronous*.

Biological systems, on the other hand, typically include choices among several pathways, as illustrated, for example, by the numerous different pathways followed by various cell lines from a zygote during embryonic development. Furthermore, it is unrealistic to assume that all time delays are equal. For example, if two genes are turned on at time t , there is no reason to expect their products to reach their threshold concentrations simultaneously. For these reasons, the synchronous description is poorly suited to biological systems.

IV. KINETIC LOGIC: AN ASYNCHRONOUS SEQUENTIAL DESCRIPTION

The formalism we have developed, called kinetic logic, introduces time in a radically different way. We borrow from Florine's sequential logic the essential notions of internal functions and variables, related by a time shift. However, our purpose was to formalize "interesting" biological systems exhibiting multiple stable states, branched pathways, and the like, so from the outset modifications were introduced to accommodate these features.

When describing gene expression, the relevant elements are, first of all, the concentrations of all regulatory substances. We thus consider these concentrations the variables and the expression (on or off) of the genes in question, the functions. Many biological regulators are the protein products of regulatory genes. The presence or absence of such a regulator is thus directly related to the state, on or off, of the corresponding gene. (Other types of regulatory molecules are synthesized by enzymes which, in turn, are proteins coded for by genes, so again the presence or absence of the regulator is simply related to gene expression.) This relationship can be illustrated as follows. Let the function X represent the state, on or off, of a gene, and the variable x represent the presence or absence of the gene product. If the state of the system is such that the gene is switched on (X commutes from 0 to 1), its product will be synthesized and, after a time delay t_x (the "on" delay), it will reach its threshold concentration (and the variable x will commute from 0 to 1). Similarly, when the gene is switched off (X commutes from 1 to 0), the product will decay or be diluted out and, after another time delay $t_{\bar{x}}$ (the "off" delay), it will drop below its threshold concentration (and x will commute from 1 to 0):



To complete the picture, we are often led to include *input variables* and *output functions* in our description of a system. The former may reflect, for example, the temperature, which can be important in the case of a temperature-sensitive (mutant) protein, or the external concentration of certain molecules, which can act as effectors of one or another regulatory protein. Output functions exert no retroaction (feedback) on the system and therefore do not require an associated variable.

Our description of a system thus consists of a set of logical formulae which relate the logical value of internal (and output) functions to the present value of internal (and input) variables.

The above illustrates the main features of our formalism and clearly shows the influence of Florine's sequential logic. It also reveals certain important differences:

1. The internal variables and functions, which are simply formal devices in Florine's logic, have become the backbone of the kinetic logic description, in which the internal variables represent the most relevant concrete elements of the system.

2. More specifically, for each element of a system, the value of the associated *internal variable* represents the *level* of the element and the value of the corresponding *internal function* represents its *evolution*. **When the logical value of a variable differs from that of the corresponding function, the variable is committed to change in the near future.** Thus, in addition to considering an internal variable as a memory of the preceding value of its associated function, we also think of an internal function as a "preview" of the forthcoming value of the variable.
3. The time delays in sequential networks are short time shifts of arbitrary duration. In kinetic logic, in view of the concrete relation between an internal function (gene on or off) and its associated internal variable (gene product present or absent), the time delays have become concrete entities whose values, far from being arbitrary, reflect specific physical processes (synthesis, degradation, dilution, etc.). In fact, the actual values of the different time delays play an important role in determining the pathway along which the system evolves (cf. Chapter 4). In the above example, the on delay t_x represents the time elapsed between turning the gene on ($X = 1$) and reaching the threshold level of the protein ($x = 1$); we can think of it as the time between the issuing of an "order" ($X = 1$) and its execution ($x = 1$). During this time, the gene must be transcribed into mRNA, and the mRNA may have to be processed. It is then translated into a polypeptide, which may have to be processed, assembled, oligomerized, etc. to form the final product, and sufficient product must accumulate before it can act as an effective regulator. Typically, these processes take several minutes. However, the efficiency of transcription and translation varies widely from one gene to another, as do the threshold concentrations of different regulators. The time delay t_x is thus specific to one particular gene and is unlikely to be equal to the on delay of another gene.

Similar considerations hold for the off delay $t_{\bar{x}}$. Here, the time between the issuing of the order " $X = 0$ " (gene off) and its execution " $x = 0$ " (product absent) will depend on the stability of the product and rate of dilution due to growth. In general, the off delays of different genes vary more than the on delays, and again any given delay $t_{\bar{x}}$ is unlikely to be equal to the off delay of another gene. For example, the positive regulatory protein N of bacteriophage λ has a half-life of about 2 min, whereas the negative regulatory protein cI (the repressor) under some conditions is so stable that it must be diluted out by growth, requiring more than a generation before falling below its threshold concentration.

In kinetic logic, the on and off delays for a given gene will generally be unequal, and the delays of different genes will also be different. Obviously, this asynchronous description is much more realistic than the synchronous description discussed above in Section III. Nevertheless, one must not lose sight of the fact that it is still a rather crude idealization of biological systems. This point will be discussed in Chapter 4 (Section IV).

V. KEY TO PART I

Part I (Chapters 2 to 8) is devoted to methods. It includes the elements of combinatorial logic required for understanding what follows (Chapter 2), various aspects of kinetic logic (Chapters 3, 4, 7, and 8), an inductive method for finding the logical structures compatible with a given pattern of biological behavior (Chapter 5), and the elements of the differential description (Chapter 6). The reader will notice that the latter is inserted between two chapters on kinetic logic; this is because the last aspect of kinetic logic (generalized description) requires a minimal understanding of the differential description.

Chapters 3 and 4 might well be called "a naïve description". In a more elaborate treatment (Chapters 7 and 8), we discuss the use of variables and functions having more than two values¹¹ and we introduce logical parameters (recently developed by Snoussi¹²) that assign a

"weight" to each term of the logical expressions. It will be seen that some of these developments are directly inspired by a comparison of the logical and differential descriptions. We comment on the similarities and differences between these two descriptions in Chapter 8.

In Chapter 5, which is perhaps the most original, we apply kinetic logic in reverse, as it were, to derive formal logical relationships capable of accounting for observed behavior. Classically, the construction of models or hypotheses to explain experimental observations has been essentially intuitive. Although our method (fortunately) does not eliminate the need for biological intuition, it provides a rational procedure for one important part of the process, viz., the elaboration of all simple logical circuits permitting or imposing a given pattern of behavior.

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Chapter 2

ELEMENTS OF COMBINATORIAL LOGIC**TABLE OF CONTENTS**

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I. BINARY VARIABLES AND FUNCTIONS

Binary variables and functions can be compared to switches (inputs) and lamps (outputs), respectively, in an electric circuit. Consider a combinatorial circuit, that is, a circuit in which each lamp is on or off depending simply on the present state of the switches. With each switch, one can associate a binary variable that takes the value 0 if the switch is off, and 1 if it is on; the state, on (1) or off (0), of each lamp is a function of the state of the switches (variables). Authoritative descriptions of combinatorial as well as sequential logic can be found in Miller,¹ McCluskey,² or Florine.³ A more popular introduction, yet more detailed than ours, can be found in Leussler and Van Ham.⁴

What has just been said of electric switches can be applied to statements, which can be true (1) or false (0), or to gene products, which can be present (1) or absent (0). For example, the following three systems are identical from the logical point of view and one should be able to formalize them in the same way: (1) a lamp which is on iff (that is, if and only if) switch a is on and switch b off; (2) a statement which is true iff statement a is true and statement b false; and (3) a gene which is on iff product a is "present" and product b "absent" ("present" means that the concentration exceeds a threshold value).

A logical function can be described by appropriate operations on the variables. The simple situation just evoked, for example, can be described by a logical function F that takes the value 1 in the situation a and not b — that is, whenever $a = 1$ and $b = 0$; otherwise, F takes the value 0. This can be represented by a "truth table", which gives the logical value of F for each combination of values of the variables (Table 1).

TABLE 1
Truth Table of a Two-Variable Function

a	b	F
0	0	0
0	1	0
1	1	0
1	0	1

II. LOGICAL OPERATORS

In this work, we will systematically use three operations: NOT, AND, and OR. As we shall see below, it is possible to describe any binary function with a *single* appropriate operator. This is extremely useful in electronics, but for our purposes, a combination of NOT, AND, and OR describes situations in a much more familiar way.

A. NOT: THE LOGICAL COMPLEMENT

The function "NOT a " has the value 1 when $a = 0$ and 0 when $a = 1$. In verbal sentences, we write "NOT a ", but in formalized logical expressions, we write " \bar{a} "; in either case, we say "NOT a ". It is obvious from Table 2 (and from the definition) that $\bar{\bar{a}} = a$, i.e., NOT (NOT a) = a .

TABLE 2
Truth Table of the NOT Operator ($F = \bar{a}$)

a	NOT a
0	1
1	0

B. AND: THE LOGICAL PRODUCT

The function "*a AND b AND c AND ...*" has the value 1 iff *a, b, c, ...* all have the value 1. In particular, "*a AND b*" has the value 1 iff *a* and *b* both have the value 1, as shown in Table 3.

TABLE 3
Truth Table of the AND Operator ($F = ab$)

a	b	a AND b
0	0	0
0	1	0
1	1	1
1	0	0

This operation is called the *logical product* by analogy with the arithmetic product: in both cases the result is nonzero only if *all* factors are nonzero. Accordingly, in formalized logical expressions, "*a AND b AND c AND ...*" is simply noted "*abc...*". This is very convenient because in calculations the logical AND behaves much like the familiar arithmetic product. Nevertheless, it is advisable to read "*abc*" as "*a AND b AND c*". The logical product is equivalent to the intersection in set theory, which is usually symbolized \cap or \wedge .

C. OR: THE LOGICAL SUM

The word "or" in English (as well as "ou" in French, "oder" in German) is used in two very different logical senses. In verbal expressions, "*a or b*" can mean "either *a* (but not *b*) or *b* (but not *a*)"; this is the *exclusive* "or". Alternatively, "*a or b*" can mean "*a* or *b* or both" or, equivalently, "*one or more of a,b*"; this is the *inclusive* "or". This regrettable ambiguity is responsible for innumerable misunderstandings, voluntary or not: false dilemmas, etc.

In commercial language, the inelegant expression "and/or" is often used to make it clear that the inclusive "or" is intended. The ambiguity has, of course, been eliminated in formalized descriptions; we use "OR" (formalized: $+$) for the inclusive "or", and say explicitly "exclusive OR" (formalized: \oplus) for the other meaning.

The function "*a OR b OR c OR ...*" has the value 1 provided at least one of its terms has the value 1, 0 otherwise. In particular, "*a OR b*" has the value 1 if *a* or *b* or both have the value 1, as shown in Table 4.

TABLE 4
Truth Table of the OR Operator ($F = a + b$)

a	b	a OR b
0	0	0
0	1	1
1	1	1
1	0	1

This operation is called the *logical sum* for its (incomplete!) analogy with the arithmetic sum. In both cases, the result is zero only if all terms are 0. Accordingly, in formalized logical expressions, "a OR b OR c OR ..." is noted " $a + b + c + \dots$ ". Again, this is very convenient because in calculations the logical OR behaves much like the familiar arithmetic sum. Nevertheless, it is advisable to read " $a + b + c$ " as " a OR b OR c". The logical sum is equivalent to the union in set theory, which is usually symbolized \cup or \vee .

These operations can be combined in various ways, and they yield all possible binary functions. The truth table of a compound function expressed in these terms can be obtained by sequentially applying in the proper order the operations NOT, AND, and OR already defined and summarized in the left part of Table 5.

TABLE 5
Truth Table of Some Two-Variable Functions

a	b	\bar{a}	\bar{b}	ab	$a + b$	\overline{ab}	$\bar{a} + \bar{b}$	$\overline{a + b}$	$\bar{a}\bar{b}$
0	0	1	1	0	0	1	1	1	1
0	1	1	0	0	1	1	1	0	0
1	1	0	0	1	1	0	0	0	0
1	0	0	1	0	1	1	1	0	0

For instance, the truth table of \overline{ab} is obtained by applying the operation NOT to column ab, and the truth table of $\bar{a} + \bar{b}$, by applying the operation OR (+) to columns \bar{a} and \bar{b} .

Let us examine some simple combinations of logical operations (right part of Table 5). It can be seen that:

$$\overline{ab} = \bar{a} + \bar{b}$$

$$\overline{a + b} = \bar{a}\bar{b}$$

This is (for two variables) a demonstration of the very important law of de Morgan.

Note that $\overline{a + b}$, i.e. NOT (a OR b), is usually contracted to NOR (a,b). The familiar expression for this operation is "neither a nor b", whose relation with the form $\bar{a}\bar{b}$ (Table 5) is perhaps more obvious. Similarly, \overline{ab} , i.e., NOT (a AND b), is usually contracted to NAND (a,b). A familiar expression is "not a and b together".

Either NOR or NAND can be used *alone* to describe *any* binary function. For example, "NOT a" is equivalent to "neither a nor a"; thus, $\bar{a} = \text{NOR } (a,a)$. Since $\bar{a}\bar{b} = \bar{a} + \bar{b}$, $ab =$

$\overline{a + b} = \text{NOR}(\text{NOR}(a,a), \text{NOR}(b,b))$. Since $\overline{a + b} = \text{NOR}(a,b)$, $a + b = \overline{\text{NOR}(a,b)} = \text{NOR}(\text{NOR}(a,b), \text{NOR}(a,b))$. This is extremely useful and extensively used in electronics because it is advantageous to use a single type of operation (a single "gate", in electronic jargon), but it is certainly less confusing for our purpose to write $(a \text{ OR } b)$ than $\text{NOR}(\text{NOR}(a,b), \text{NOR}(a,b))$!

How many different logical functions can one build with two binary variables? Clearly, a function may have two possible values (0 or 1) for each of the four states (00, 01, 11, 10) of the variables. There are thus $2^4 = 16$ distinct logical functions of two binary variables. More generally, for n binary variables there are 2^n states (combinations of values) and 2^{2^n} different functions; and with a logic using k -valued variables and functions, there are k^{k^n} different functions of n variables. We have already seen eight functions of the two binary variables "a" and "b":

$$F = a, F = b, F = \bar{a} \text{ (not A)}, F = \bar{b} \text{ (not b)}, F = ab \text{ (a AND b)},$$

$$F = a + b \text{ (a OR b)}, F = \overline{a + b} \text{ (NOR (a,b))}, F = \overline{ab} \text{ (NAND (a,b))}.$$

Some other functions of two variables are given in Table 6. As noted at the lower part of the table, all these functions have a familiar meaning. Function $\bar{a}b + a\bar{b}$ is, of course, the *exclusive OR*, often symbolized $a \oplus b$. This function has the value 1 iff a and b have different values; it is thus equivalent to $a \neq b$. Function $ab + \bar{a}\bar{b}$ is the complement of the exclusive OR (see the truth table). This function has the value 1 iff a and b have the same value; it is thus equivalent to $a = b$ (*identity*). Function $\bar{a} + b$ (or, equivalently, $\overline{\bar{a}b}$) is not satisfied ($F = 0$) when $a = 1$ and $b = 0$; it is thus equivalent to the *implication* $a \rightarrow b$ (if a , then b ; in other words, if $a = 1$, one cannot have $b = 0$). Similarly, $a + \bar{b}$ (or $\overline{a\bar{b}}$) is equivalent to the implication $b \rightarrow a$.

TABLE 6
Truth Table of Some Additional Two-Variable Functions

a, b	$\bar{a}b + \bar{a}\bar{b}$	$ab + \bar{a}\bar{b}$	$\bar{a} + b$	$a + \bar{b}$
00	0	1	1	1
01	1	0	1	0
11	0	1	1	1
10	1	0	0	1
Name of function	Exclusive OR	Identity	Implication ($a \rightarrow b$)	Implication ($b \rightarrow a$)
Various expressions for the function	$a \oplus b$	$\overline{a \oplus b}$	$\overline{\bar{a}b}$	$\overline{a\bar{b}}$
	$a \neq b$	$a = b$	$\bar{a} + b$	$a + \bar{b}$

III. DIFFERENT EXPRESSIONS OF A LOGICAL FUNCTION

It is apparent from the preceding paragraphs that a logical function can be given several formal expressions, not only by using different logical operators, but even with a given choice of operators. For instance, a implies b can be written:

$$a \rightarrow b$$

$$\left. \begin{array}{l} \overline{a\bar{b}} \\ \bar{a} + b \\ \bar{a}\bar{b} + \bar{a}b + ab \end{array} \right\} \begin{array}{l} \text{Three different expressions within the "system"} \\ \text{NOT, AND, OR} \end{array}$$

The last version is a direct expression of the truth table. It says that the function is satisfied by three states of the variables: 00 ($\bar{a}\bar{b}$), 01 ($\bar{a}b$), and 11 (ab). Any function can be expressed in this way, that is, as the sum of the individual states which give the function the value 1 (these are called "minterms" in logical jargon). However, it is clear that in most cases this representation will be rather long and not illustrative.

This raises the important question: how can one simplify a logical expression to render it as compact as possible? For this, we will use Veitch⁶ (or Karnaugh⁷) maps, which are another presentation of truth tables. For two, three, and four variables, the inputs of these maps are disposed as follows (Table 7).

TABLE 7
Two-, Three-, and Four Variable Truth Tables^{6,7}

F	0	1	a
0	•	•	
1	•	•	
b			

F	00	01	11	10	a, b
0	•	•	•	•	
1	•	•	•	•	
c					

F	00	01	11	10	a, b
00	•	•	•	•	
01	•	•	•	•	
11	•	•	•	•	
10	•	•	•	•	
c, d					

To each combination of values of the variables ("state") there corresponds a square in the table. There are thus 2^2 , 2^3 , and 2^4 squares for two, three, and four variables, respectively. In each square, one writes the corresponding value(s) of the function(s). Initially, we will use only one function (F) per table.

Note that the way the input values are presented (00, 01, 11, 10), two adjacent columns or lines differ by the value of one variable only, and the first and last columns or lines behave as adjacent in this respect; in other words, the map is a torus. This order (called "Gray code" order) is extremely convenient, as will be seen below.

Consider now the function $F1 = \bar{a}\bar{b} + \bar{a}b + ab$. In this form, each term corresponds to an elementary square of the map (Table 8A). One could, however, combine two elements: $F1 = \bar{a}(\bar{b} + b) + ab = \bar{a} + ab$. Now the first term corresponds to the fusion of two adjacent squares in the map (Table 8B).

TABLE 8
Maps Suggesting Various Expressions of a Function

F1	0	1	a
0	1	0	
1	1	1	
b			

A

F1	0	1	a
0	1	0	
1	1	1	
b			

B

F1	0	1	a
0	1	0	
1	1	1	
b			

C

F1	0	1	a
0	1	0	
1	1	1	
b			

D

$\overline{F1}$	0	1	a
0	0	1	
1	0	0	
b			

E

There is another way to fuse two elements of the initial expression: $F1 = \overline{a}b + b(\overline{a} + a) = \overline{a}b + b$. This is illustrated by Table 8C. Finally, using a trick which consists of writing the term $\overline{a}b$ twice, one can write $F1 = \overline{a}b + \overline{a}b + \overline{a}b + ab = \overline{a}(\overline{b} + b) + b(\overline{a} + a) = \overline{a} + b$. This is illustrated by Table 8D.

At first, one would be tempted to say that notation D is redundant because, as one sees on the map, element $\overline{a}b$ is "covered" twice; it is present in term \overline{a} and in term b . In fact, this is the most compact of all four notations just presented.

In some cases, \overline{F} can be expressed as (or almost as) simply as F itself. For instance, here, $\overline{F1}$, obtained by replacing the 0's with 1's and vice versa in the map of $F1$, is shown in Table 8E. The expression of $\overline{F1}$ is $\overline{a}b$, from which one can derive another simple expression of $F1$: $F1 = \overline{\overline{a}b}$, which, according to the de Morgan rule, is equivalent to $F1 = \overline{a} + b$.

Let us now try to generalize the method of simplification.

1. Adjacent terms can be joined two by two, four by four, eight by eight, etc., using the general principle: $\overline{a}b + \overline{a}b = \overline{a}(\overline{b} + b) = \overline{a}$. This can be done by calculus; however, with a little practice it is incomparably easier to use Karnaugh maps. Let us consider four simple examples.

$$\begin{aligned}
 F2 &= \overline{a}\overline{b}\overline{c}\overline{d} + \overline{a}b\overline{c}\overline{d} + \overline{a}\overline{b}cd + \overline{a}bcd \\
 F3 &= \overline{a}\overline{b}\overline{c}\overline{d} + \overline{a}b\overline{c}\overline{d} + \overline{a}b\overline{c}d + \overline{a}bcd \\
 F4 &= \overline{a}\overline{b}\overline{c}\overline{d} + \overline{a}b\overline{c}\overline{d} + \overline{a}b\overline{c}d + \overline{a}bcd \\
 F5 &= \overline{a}\overline{b}\overline{c}\overline{d} + \overline{a}b\overline{c}\overline{d} + \overline{a}b\overline{c}d + \overline{a}bcd
 \end{aligned}$$

A look at the Karnaugh maps (Table 9) shows that in each case the four terms can be fused into a unique term (the grouping is at first less obvious for F4 and F5, but one must remember that the extreme lines and columns of the map are adjacent).

TABLE 9
Simplification of Functions

F2	00	01	11	10	ab
00	1	1	0	0	
01	1	1	0	0	
11	0	0	0	0	
10	0	0	0	0	
cd					

F3	00	01	11	10	ab
00	1	1	1	1	
01	0	0	0	0	
11	0	0	0	0	
10	0	0	0	0	
cd					

F4	00	01	11	10	ab
00	0	0	0	0	
01	1	0	0	1	
11	1	0	0	1	
10	0	0	0	0	
cd					

F5	00	01	11	10	ab
00	1	0	0	1	
01	0	0	0	0	
11	0	0	0	0	
10	1	0	0	1	
cd					

Thus,

$$F2 = \bar{a} \bar{c}$$

$$F3 = \bar{c} \bar{d}$$

$$F4 = \bar{b} d$$

$$F5 = \bar{b} \bar{d}$$

Note that (1) this grouping is greatly facilitated by the use of the Gray code order and (2) the more elementary terms one can group, the simpler the expression; $abcd$ covers one minterm; abc , two; ab , four; and a , eight.

A term that cannot be condensed with any other term is called a *prime implicant*. To be as compact as possible, the final expression should contain prime implicants *only*. A function can always be expressed as the sum of all its prime implicants. In the above examples, this gives the most compact expression; however, as we shall see, *not all* prime implicants are necessarily needed to describe the function, that is, to cover each "1" square and none of the "0" squares. Consider, for instance: $F6 = \bar{a}c + \bar{a}bd + \bar{a}b\bar{d} + a\bar{c}d + b\bar{c}d + \bar{b}cd$, in which each term is a prime implicant.

TABLE 10
Prime Implicants

F6	00	01	11	10	ab
00	0	0	0	0	
01	0	1	1	1	
11	1	1	0	1	
10	1	1	0	0	
cd					

All

F6	00	01	11	10	ab
00	0	0	0	0	
01	0	1	1	1	
11	1	1	0	1	
10	1	1	0	0	
cd					

Sufficient

In Table 10 (left) are shown all prime implicants of function F6. In Table 10 (right), it can be seen that three of these prime implicants are sufficient to cover the function. We write:

$$F6 = \bar{a}c + a\bar{b}d + b\bar{c}d$$

One way to find this “minimal covering” of the function consists of first identifying the so-called *essential* prime implicants. An essential prime implicant comprises at least one “1” not present in any other prime implicant. Consider function F7 (Table 11).

TABLE 11
Prime Implicants of F7

F7	00	01	11	10	ab
00	0	0	0	0	
01	1*	1	1	1	
11	0	1	1	1*	
10	1*	1	0	0	
cd					

All

F7	00	01	11	10	ab
00	0	0	0	0	
01	1	1	1	1	
11	0	1	1	1	
10	1	1	0	0	
cd					

Essential

F7	00	01	11	10	ab
00	0	0	0	0	
01	1	1	1	1	
11	0	1	1	1	
10	1	1	0	0	
cd					

Minimal covering

The three 1's labeled * (Table 11, top left) are each present in only one prime implicant. There are thus three essential prime implicants, which are represented in Table 11, middle. As nothing is perfect, there is a “1” ($\bar{a}bcd$) that is covered by none of the essential prime

implicants. To take this into account, we will add the largest (and, consequently, simplest) prime implicant which comprises this state; it is obviously bd . Thus, we write:

$$F7 = ad + bd + \bar{c}d + \bar{a}\bar{c}\bar{d}$$

IV. INCOMPLETELY SPECIFIED MAPS

It often happens that, for certain input states, it does not matter whether the output is 0 or 1. There may also be input states which correspond to physically impossible situations. As these input states will not occur in reality, one can assign the function a value of 0 or 1 at will. These unspecified states are represented by a dash on Karnaugh maps. Since each dash can be replaced by a 1 or a 0, a map with n dashes represents a set of 2^n functions, of which one can choose the most convenient (usually the simplest).

TABLE 12
An Incompletely Specified Map

F	00	01	11	10	ab
00	0	0	1	0	
01	1	-	-	0	
11	1	-	-	0	
10	0	0	0	-	
cd					

F	00	01	11	10	ab
00	0	0	1	0	
01	1	1	1	0	
11	1	1	0	0	
10	0	0	0	0	
cd					

Consider, for example, the map of Table 12 (left). There are five dashes, thus $2^5 = 32$ functions consistent with this map. With a little practice, one immediately finds the simplest one, suggested in Table 12 (left) and given explicitly in Table 12 (right):

$$F = \bar{a}d + abc$$

This technique can be conveniently used for functions of up to five or six variables. A specialized logic machine conceived and realized by Florine³ can be used for incompletely specified maps of up to eight variables. More recently, computer programs that can handle up to 100 variables have been described.⁸

V. A COMMENT ON IMPLICATION AND ON NECESSARY VS. SUFFICIENT CONDITIONS

Since $a \rightarrow b$ is equivalent to $\bar{a}b$, or $\bar{a} \vdash b$, it follows that the implications in the left column of Table 13 are equivalent, respectively, to the logical expressions in the right column.

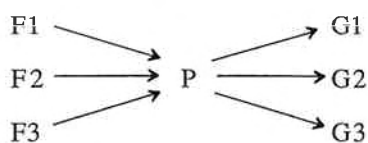
TABLE 13
Implication and Necessary vs. Sufficient Conditions

The implication	is equivalent to
$a \rightarrow b$	$\overline{a}\overline{b}$, or $\overline{a} + b$
$\overline{b} \rightarrow \overline{a}$	$\overline{a}\overline{b}$, or $\overline{a} + b$
$b \rightarrow a$	$\overline{a}b$, or $a + \overline{b}$
$\overline{a} \rightarrow \overline{b}$	$\overline{a}b$, or $a = \overline{b}$
and $(a \rightarrow b) \bullet (b \rightarrow a)$	$ab + \overline{a}\overline{b}$ or $a = b$

The notation using the NOT, AND, and OR operators thus gives an immediate demonstration that the expression $a \rightarrow b$ is equivalent to its contrapositive $\overline{b} \rightarrow \overline{a}$ and that $b \rightarrow a$ is equivalent to $\overline{a} \rightarrow \overline{b}$.

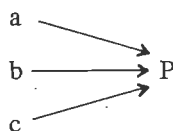
If one has both $(a \rightarrow b)$ and $(b \rightarrow a)$, then a is equivalent to b , which means that a and b must have the same logical value in all cases.

If one now writes:



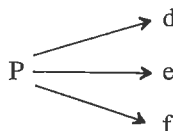
each of the F s is a *sufficient* condition for P since $F \rightarrow P$ means "if F then P ", and each of the G s is a *necessary* condition for P since $P \rightarrow G$ is equivalent to $\overline{G} \rightarrow \overline{P}$, which means that if G is not satisfied, then neither is P . This presentation of the relation between sufficient and necessary conditions is probably not original. One of its merits is to show clearly that any necessary condition is implied by any sufficient condition (a truism, alas?).

If $P = a + b + c$,



a , b , and c are each sufficient conditions for P .

If $P = def$,

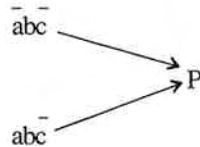


d , e , and f are each necessary conditions for P .

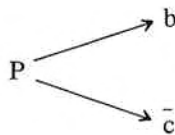
Let $P = b\bar{c}$ (1)

$$= \bar{a}b\bar{c} + a\bar{b}\bar{c}$$
 (2)

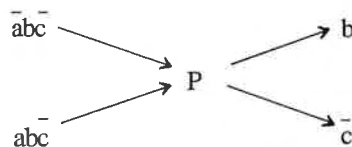
From Equation 2 one can write:



and from Equation 1:



Thus,



These remarks may seem simplistic. Nevertheless, we feel they may be useful in Chapter 5 when we consider the conditions that *permit* and the conditions that *impose* a given pattern of behavior.

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KINETIC LOGIC I: FROM LOGICAL STRUCTURES TO BEHAVIOR

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I. LOGICAL VARIABLES AND FUNCTIONS

Classically, the evolution of a logical system with time has been described by relating the value of the variables at time $t + 1$ to their value at time t . As already mentioned, this description gives each state only one potential follower, and its synchronous character makes it unsuitable for biological systems.

We avoid this problem¹⁻³ by associating with each relevant element of our systems not only a *logical variable* (x, y, z, \dots) that represents the *level* (e.g., concentration) of the element, but also a *logical function* (X, Y, Z, \dots) whose value reflects the *evolution* (e.g., rate of synthesis) of the element. We write $X = \Phi_1(x, y, z, \dots)$, in which Φ_1 is a logical function describing the influences affecting the evolution of variable x .

Concretely, we will most often deal with gene expression. In *simple* cases, we reason as though the gene products were present or absent and the gene on or off. Thus,

$x = 0$ means "gene product absent"

$x = 1$ means "gene product present"

and

$X = 0$ means "gene off"

$X = 1$ means "gene on"

To avoid confusion between an *object* and the variables or functions associated with it, we symbolize the object by an *underlined* letter: we call \underline{X} the gene and \underline{x} its product. For logical functions and variables, we use boldface italics: X and x .

Suppose that gene \underline{X} is on iff product \underline{z} is absent and that gene \underline{Y} is on iff \underline{z} is absent and \underline{u} is present. We write simply:

$$\begin{aligned} X &= \bar{z} \\ Y &= \bar{z}u \end{aligned} \tag{1}$$

Such relations express the state, on or off, of a gene *at any time* according to the present values of the variables. How, then, is time included? Simply because the presence of a gene product *now* implies that the corresponding gene was on at *some earlier time*. There is thus a circular relation between our variables and functions in the sense that the value of each function depends on the present value of the variables, whereas the value of each variable depends on earlier values of the *corresponding* function. This circular relation between variables and functions is not a peculiarity of the logical description. When a system is described by a set of differential equations such as

$$\frac{dx}{dt} = H_x(x, y, z)$$

the value of the function H_x depends on the present values of the variables x, y, z, \dots , but, in turn, this present value of x depends on earlier values of dx/dt .

The temporal relation between a logical variable x associated with the *level* of an element and a logical function X associated with its *evolution* can be illustrated as follows. Consider a gene that has been off ($X = 0$) for a considerable time, then is switched on ($X = 1$) by a signal (in the present case, the disappearance of product \bar{z}), and then, after some time, is switched off again ($X = 0$) by another signal (here, the reappearance of product \bar{z}) (see Figure 1). How will x vary with time? When the gene has been off for a sufficient time, its product, which is perishable, will be absent ($x = 0$). When the gene is switched on ($X = 1$), the product will appear, but not immediately; $X = 1$ (gene already on), but $x = 0$ (product still absent) until a proper delay t_x has elapsed. From then on, $X = 1$ and $x = 1$ (gene on, product present) as long as the gene is on. But if a signal switches the gene off ($X = 0$) temporarily, $X = 0$ and $x = 1$ (gene off, but product still present) until the product has disappeared; this requires a time delay $t_{\bar{x}}$, after which once again $X = 0$ and $x = 0$.

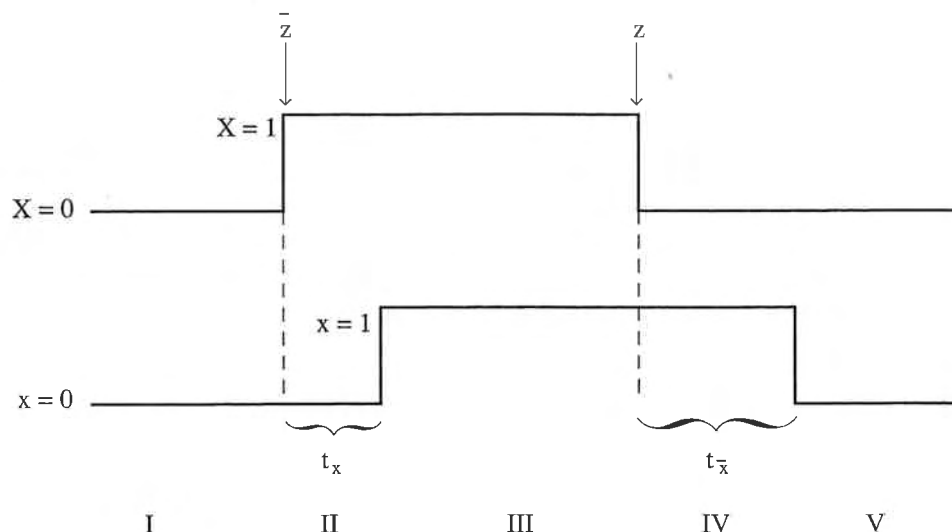


FIGURE 1.

As discussed in Chapter 1, the time delays t_x and $t_{\bar{x}}$ have no reason to be the same; t_x depends principally on the rate of synthesis of \bar{x} , whereas $t_{\bar{x}}$ depends to a large extent on the stability of \bar{x} . A gene product can reach an effective concentration a few minutes after the gene has been switched on, but remain active for hours after the gene has been switched off.

In the example chosen (1), the same signal (the disappearance of product \bar{z}) may switch on genes \bar{X} and \bar{Y} . Let us start from a situation in which products \bar{x} and \bar{y} are absent and products \bar{z} and \bar{u} are present. If, now, product \bar{z} suddenly disappears, the two genes will be switched on simultaneously: $\bar{X} = 1$ and $\bar{Y} = 1$. However, the genes have different rates of transcription and translation, the specific activities and threshold levels of their products are different, etc. There is no reason why product \bar{x} and \bar{y} should reach their threshold value simultaneously. Thus, if the two genes remain on ($\bar{X} = 1$, $\bar{Y} = 1$), the products \bar{x} and \bar{y} will eventually appear ($\bar{x} = 1$, $\bar{y} = 1$), but after *different* time delays ($t_x \neq t_y$).

In practice, we normally ascribe two distinct time delays ("on" and "off") to each element of the system: t_x , $t_{\bar{x}}$, t_y , $t_{\bar{y}}$, etc. More generally, a characteristic time delay is ascribed to each *transition* of the logical system. Suppose, for instance, that a particular gene product can disappear either because the gene has simply been switched off or because a protease that specifically destroys this gene product has appeared. The "off" delays will presumably be very different in these two situations.

Before proceeding further, let us again consider Figure 1. In periods I and V, the gene is off ($X = 0$) and the product absent ($x = 0$): nothing is likely to change as long as the gene remains off. In period III, the gene is on ($X = 1$) and the product present ($x = 1$): again, nothing should happen as long as the gene remains on. In contrast, in period II, the gene has just been switched on ($X = 1$); the product is still absent ($x = 0$), but, if the gene remains on, the product will eventually appear. Similarly, in period IV, the gene has just been switched off ($X = 0$); the product is still present ($x = 1$) but (if the gene remains off) it will eventually disappear. These two periods are inherently transient.

In general, as long as a function and the corresponding variable have the same logical value (gene off and product absent: [$X = 0, x = 0$] or gene on and product present: [$X = 1, x = 1$]), no change is expected to take place. However, when the values of the function and variable momentarily "disagree" (gene on, but product still absent, or gene off, but product still present), there is an *order* for the variable to align its value with the new value of the function, and this order will be executed after a delay unless there is a counterorder (a new change of the value of the function) before the delay has elapsed.

II. NAÏVE LOGICAL DESCRIPTION

Let us first examine a simple two-element system. Suppose that product x activates gene Y and product y represses gene X . In other words,

$$X = 1 \text{ iff } y = 0 \text{ (}\underline{X} \text{ "on" iff } y \text{ absent)}$$

$$Y = 1 \text{ iff } x = 1 \text{ (}\underline{Y} \text{ "on" iff } x \text{ present).}$$

This can be described by the graph of interactions $x \begin{array}{c} \xrightarrow{+} y \\ \xleftarrow{-} x \end{array}$ (see Section IV.A) As

we will see in Chapter 9, feedback loops are of two types, positive or negative, according to whether there is an even or odd number of negative interactions. In technical language, our circuit is a simple negative loop.

The logical relation in this system can be written:

$$X = \bar{y}$$

$$Y = x$$

and tabulated

x	y	X	Y
0	0	1	0
0	1	0	0
1	1	0	1
1	0	1	1

The left part of the table is simply a repertoire of the 2^n possible states of the variables. A convenient way to complete the right part is to fill it in *column by column*:

$$X = 1 \text{ for each state for which } y = 0;$$

$X = 0$ otherwise, etc...

This *state table* says, for each state of the variables (\bar{x} , \bar{y} present or absent), which products are and which are not being synthesized at a significant rate (in genetics, which genes are on and which are off). It is convenient to treat the state of the variables as a logical vector xy , which can take the values 00, 01, 11, 10, and the state of the logical functions as a vector XY .

In the system:

$$X = \Phi_1(x, y, \dots)$$

$$Y = \Phi_2(x, y, \dots)$$

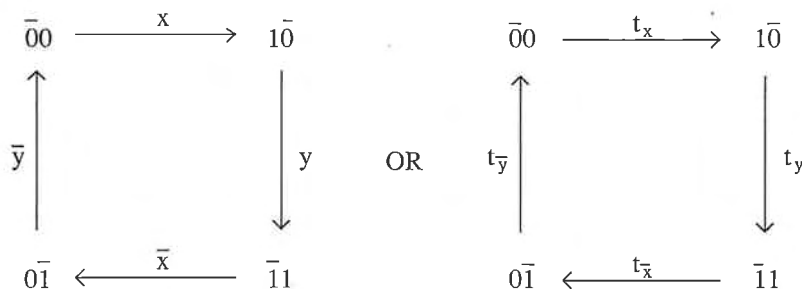
vector $XY \dots$ is obtained by applying operation Φ to vector $xy \dots$; for this reason, vector $XY \dots$ is called the *image* of vector $xy \dots$. In fact, *this image $XY \dots$ corresponds to the state that would be reached if all the orders applied to state $xy \dots$ were executed.* As we will see (for example, at the end of this section), the image $XY \dots$ is *usually not* the next state, if only because not all orders are executed at once.

The "total" state of the system is thus given by xy/XY (for instance, 00/10); however, there is a more compact notation. Consider, for instance, the state 00/10, in which both gene products are absent but gene \bar{X} is on. As product \bar{x} is absent but being synthesized, it can be expected that in the near future it will be present and, consequently, that the logical value of variable x will change from 0 to 1. This can be described by the notation $\bar{0}0$, in which the dash above the first digit draws attention to the fact that variable x is committed to change its value from 0 to 1. *More generally, we put a dash over the figure representing the logical value of a variable each time this value is different from that of the corresponding function.*

The state just considered can thus be represented as 00/10 or, more compactly, $\bar{0}0$. The notation $\bar{0}0/10$ is redundant, but convenient in state tables. Thus, we rewrite the state table:

x	y	X	Y
$\bar{0}$	0	1	0
0	$\bar{1}$	0	0
$\bar{1}$	1	0	1
1	$\bar{0}$	1	1

From this table, it is easy to derive the temporal sequence of states of the system. We write:



depending on whether we want to insist on the transitions x , y , \bar{x} , \bar{y} , or on their duration t_x , etc. We call this representation a *graph of sequence of states* (see Section IV.B). Note that in this very simple case there is a single dash for each state; this means that the variables are called to change their value one at a time. Clearly, the sequence of states would be the same whatever the values of the time delays; this is not so in most cases (see for example the following section).

Those familiar with the differential description may be surprised to see that a *Boolean* two-element negative circuit displays *stable periodic* behavior, whereas a *differential* system of the same structure tends toward a *stable steady state* (see Chapter 6). There is, in fact, no contradiction. For parameter values which ensure efficient homeostasis, the steady state (a "stable focus") is approached periodically, following a damped oscillation. Damping, however, is slower and slower as sigmoid interactions become steeper, and, as they approach step functions, the system will oscillate indefinitely. Furthermore, by introducing appropriate time delays in the differential equations, one can obtain stable periodic behavior, even for finite steepness with two-element (and even one-element) negative loops.

It is important to realize the basic similarity between the stable cycle predicted by the logical description and the stable focus predicted (for proper parameter values) by the differential description. In both cases homeostasis is achieved, i.e., the variables either *oscillate around* or *stabilize at* a value intermediate between their lowest and highest values, which would correspond to the states in which the gene is permanently off or on, respectively. The situation could be sketched by saying that each variable oscillates around its threshold. In many cases, they will stabilize at this intermediate level, as if the gene were half on, half off.

As a second example, we will analyze a two-element positive loop: $x \xrightarrow{+} y \xrightarrow{+} x$,
for instance, two genes each of which is repressed by the product of the other.

In the naïve logical description, we write:

$$X = \bar{y}$$

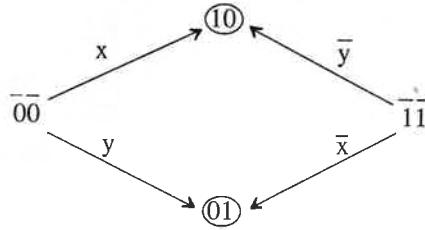
$$Y = \bar{x},$$

with the following state table:

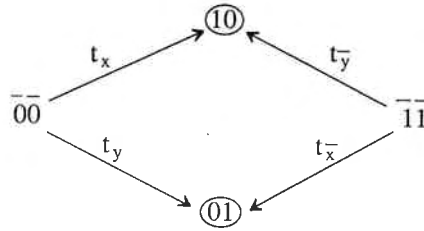
x	y	X	Y
$\bar{0}$	$\bar{0}$	1	1
0	1	0	1
$\bar{1}$	$\bar{1}$	0	0
1	0	1	0

Here, there are two states, 01 and 10, for which the vectors xy and XY have the same value. In the first case, \bar{x} is absent and not being synthesized, y is present and being synthesized. There is thus no reason why the situation would change and, accordingly, we have a logical *stable state*, and we write $\textcircled{01}$ (and similarly, $\textcircled{10}$). More generally, we define logical stable states as those for which the vectors $xy \dots$ and $XY \dots$ are equal.

What about the two other states of this system? In state $\bar{0}\bar{0}$, both products are absent but both genes are on, whereas in state $\bar{1}\bar{1}$, both products are present but both genes are off. In either case, *both* variables are called to change their value. Thus, in striking contrast to the situation described in Section 1 (in which each logical state had only one possible next state), here, state $\bar{0}\bar{0}$ will be followed by $\textcircled{10}$ or $\textcircled{01}$ according to whether x or y reaches its threshold value first; and state $\bar{1}\bar{1}$ will be followed by state $\textcircled{01}$ or $\textcircled{10}$ according to whether x or y is the first to fall below its critical concentration. A double transition $\bar{0}\bar{0} \rightarrow \bar{1}\bar{1}$ (or vice versa), is conceivable. We do not take it into account explicitly, but if the two commutations happened to take place simultaneously, this event would automatically be reflected in the simulations (see Chapter 4). The overall evolution is thus:



In terms of time delays, the choices can be described as follows: from $\bar{0}\bar{0}$, the system will proceed to state $\textcircled{10}$ or to state $\textcircled{01}$, according to whether $t_x < t_y$ or $t_y < t_x$, and from $\bar{1}\bar{1}$ the system will proceed to state $\textcircled{10}$ or to state $\textcircled{01}$, according to whether $t_{\bar{y}} < t_{\bar{x}}$ or $t_{\bar{x}} < t_{\bar{y}}$



A first remark is that even simple systems can exhibit “multistationarity”, i.e., have a structure such that they can reach and persist in either of two (or more) steady states. As we shall see in more detail in Chapter 12, this is, in fact, a general property of positive loops (even those comprising only one element!). A second, more general remark is that a given logical state may have more than one potential successor. If one considers only single variable transitions, a state in an n -variable system may have up to n different potential successors. Here, the image of state $\bar{0}\bar{0}$ is $\bar{1}\bar{1}$, but the state following $\bar{0}\bar{0}$ will be either $\textcircled{10}$ or $\textcircled{01}$; similarly, the image of $\bar{1}\bar{1}$ is $\bar{0}\bar{0}$, but the state following $\bar{1}\bar{1}$ will be either $\textcircled{01}$ or $\textcircled{10}$.

III. INPUT VARIABLES

In addition to the “internal” variables considered so far, there are so-called “input” variables whose value can be decided by the experimenter and in *some* cases changed at will. For instance, in a thermosensitive process, the temperature is considered “low” ($t = 0$) or “high” ($t = 1$), according to whether it is below or above the sensitivity threshold of the process; we can impose $t = 0$ or $t = 1$ from outside.

There is also a class of input variables which is typically genetic. These are variables associated with the genetic state of the organism. When we write $X = \bar{z}$, meaning that \underline{X} is active *if* \underline{z} is absent, we implicitly assume that gene \underline{X} is normal and normally controlled. However, gene \underline{X} might be mutationally inactivated, thus yielding an inefficient product. To formalize this, we introduce a variable g_x (for gene) which takes the value 1 or 0, according to whether the gene is normal or mutationally inactivated. On the other hand, the operator, which is the site at which product \underline{z} represses gene \underline{X} , may also be mutationally inactivated. According to the case, we write $o_x = 1$ or $o_x = 0$. Thus, when we envisage using mutants in which gene \underline{X} or its operator are inactive, instead of $X = \bar{z}$, we write,

$$X = g_x(\bar{z} + \bar{o}_x),$$

which means that gene \underline{X} is active iff it is genetically normal AND (product \underline{z} is absent OR the operator is inactive). We choose in advance to use this or that allelic form (except in experiments during which such mutants are generated). Thus, g_x and o_x are input variables.

It is convenient to use different *lines* for the combinations of values of the *internal* variables and different *columns* for the combinations of values of the *input* variables. As long as the input variables do not change, the system evolves within the same column (see below), but each time the value of an input variable changes, the system migrates to a new column.

Consider, for example, the system $X = \bar{y}$, $Y = \bar{x}$, briefly analyzed in Section II, and suppose that in the experiments we are performing there may be a mutation inactivating the operator of gene \underline{X} and that gene \underline{X} carries a mutation that renders its product thermosensitive. In this case, we reason that the expression of gene \underline{X} is not dependent on temperature, but that its product is active or not according to whether the temperature is low ($t = 0$) or high ($t = 1$).

$$X = \bar{y} + \bar{o}$$

$$Y = \bar{x} + t$$

The first means that the expression of gene \underline{X} requires the absence of product \underline{y} or of the normal operator (the site of action of \underline{y}), and the second relation means that the expression of gene \underline{Y} requires that the product \underline{X} be absent or thermally denatured.

As for the input variables, there are four situations, according to whether the operator of gene \underline{X} is functional or not and the temperature low or high. This can be tabulated:

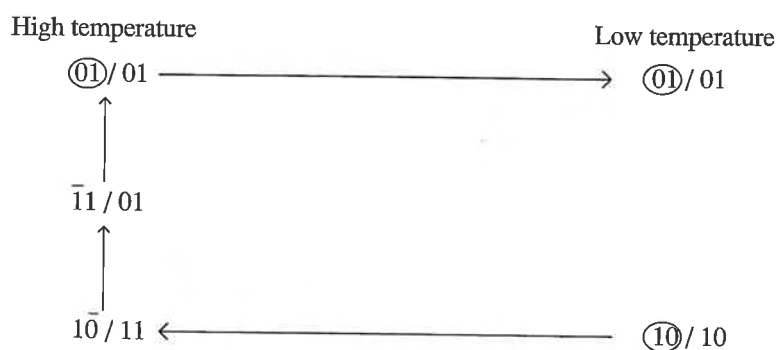
XY	00	01	11	10	o_x, t
00	11	11	11	11	
01	11	11	(01)	(01)	
11	10	(11)	01	00	
10	(10)	11	11	(10)	
xy					

↑
↑
↑
"Normal" situation:
normal operator, low
temperature.

It is, however, probably more convenient, if less elegant, to write:

$Q_x=0$ $t=0$		$Q_x=0$ $t=1$		$Q_x=1$ $t=1$		$Q_x=1$ $t=0$	
xy	XY	xy	XY	xy	XY	xy	XY
$\bar{0}\bar{0}$	11	$\bar{0}\bar{0}$	11	$\bar{0}\bar{0}$	11	$\bar{0}\bar{0}$	11
$\bar{0}1$	11	$\bar{0}1$	11	$\textcircled{0}1$	01	$\textcircled{0}1$	01
$\bar{1}\bar{1}$	10	$\textcircled{1}\bar{1}$	11	$\bar{1}1$	01	$\bar{1}\bar{1}$	00
$\textcircled{1}0$	10	$\bar{1}\bar{0}$	11	$\bar{1}\bar{0}$	11	$\textcircled{1}0$	10

In practice, what we can change *during* an experiment is the *temperature*. Let us consider the two cases on the right: operator present, low or high temperature. In the first case (column 4), we have two stable states, $\textcircled{0}1$ and $\textcircled{1}0$, whereas in the second case (column 3), we have only one, $\textcircled{0}1$. Starting from state $\textcircled{1}0/10$ in column 4, let us first shift to high temperature. Formally, this amounts to switching to column 3. We are in state $\bar{1}\bar{0}/11$, from which we will proceed to $\bar{1}1/01$ and $\textcircled{0}1/01$, where the system stabilizes. If we now return to low temperature, we go from $\textcircled{0}1/01_{t=0}$ to $\textcircled{0}1/01_{t=1}$ and remain there. The overall pathway was:



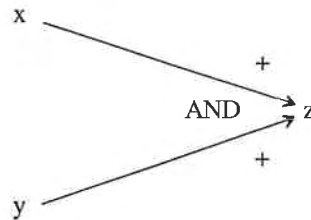
Thus, a transient change from low to high temperature has resulted in a switch from state $\textcircled{1}0$ to state $\textcircled{0}1$, and the new situation persists stably even after the return to low temperature.

IV. GRAPHS

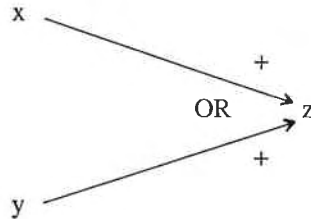
A. GRAPHS OF INTERACTIONS

The symbol $x \longrightarrow +z$ means that z is synthesized efficiently iff x is present: $Z = x$.

The graph



means that \underline{Z} is synthesized iff \underline{x} and \underline{y} are both present, i.e., $Z = xy$. The graph



means that \underline{z} is synthesized iff \underline{x} or \underline{y} or both are present. In other words, either \underline{x} or \underline{y} suffices: $Z = x + y$.

In the same way, $x \longrightarrow \neg z$ means that \underline{Z} is synthesized iff \underline{X} is absent: $Z = \bar{x}$; and similarly, for multiple interactions connected by AND or OR.

Using this symbolism, we can represent the interactions of a system by a *graph of interactions* in which each relevant element in the system forms a *vertex* and each interaction between elements forms an *edge*.

In classical graph theory, an edge is or is not drawn between two vertices, according to whether or not the corresponding elements interact. However, in many fields one has to distinguish between an influence acting from \underline{x} to \underline{y} and an influence acting from \underline{y} to \underline{x} . Accordingly, oriented edges (arrows) are used: $x \rightarrow y$ or $y \rightarrow x$. For a reciprocal interaction,

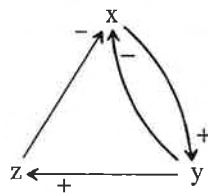
we write $x \rightleftarrows y$. This is an *oriented graph*.

In addition, as seen in the preceding graphs, we have to indicate whether an interaction is positive or negative. For example, if \underline{x} exerts a positive action on \underline{y} and \underline{y} exerts a negative

action on \underline{x} , we write $x \rightleftarrows^+ y$. This is an *oriented, signed graph*. Finally, as also

mentioned, we state whether the connection between two interactions is AND or OR.

Any closed pathway in an oriented graph is called a *circuit*. The word *loop* is often reserved for a one-element circuit, that is, for the case of an element that acts directly on its own synthesis. However, as already mentioned, the expression feedback loop is so widely used in biology that we will generally use the word *loop* instead of *circuit*.



The system shown above comprises two *circuits*, although we will call them *loops*.

B. GRAPH OF SEQUENCES OF STATES

Logical analysis provides us with the sequences of logical states which can occur, starting from any chosen initial state. This is also an oriented graph, although quite distinct from the graph of interactions. Whereas in the graph of interactions the vertices represent the elements of the system and the edges (arrows) represent the interactions between elements, in the graph of sequences of states the vertices represent the logical states of system and the edges (arrows) represent the transitions between states.

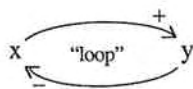
Thus, graphs of interactions are static: they indicate which interactions can take place, independently of time. Graphs of sequences, on the other hand, are dynamic: they indicate the temporal order of appearance of the logical states. As mentioned above, in graphs of interactions we call circuits "loops"; in graphs of sequences of states, we call them "cycles", which evokes an appropriate dynamic image.

For example, if we analyze the system whose graph of interactions is the two-element loop

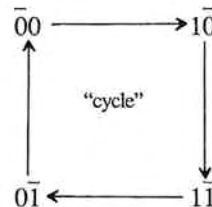


we find that the graph of sequences of states comprises a cyclic sequence of four states.

Graph of interactions



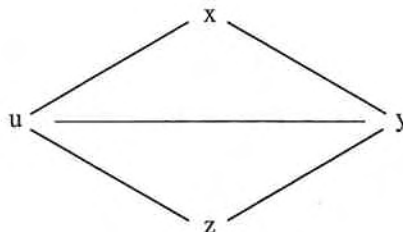
Graph of sequences of states



Classically, both graphs would be called "circuits".

C. ADJACENCY MATRICES (OR MATRICES OF INTERACTIONS)

Graphs can also be represented by so-called "adjacency matrices", or matrices of interactions. Consider, for example, the graph:

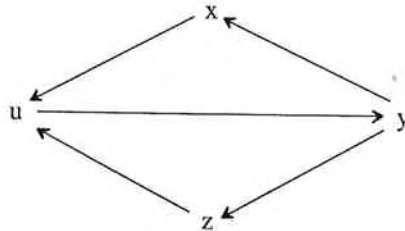


We can build the following matrix in which 1 or 0 at a given position indicates that those two elements do or do not interact. It is called an adjacency matrix because two interacting

vertices of the graph are considered adjacent. The matrix shows that in this system all pairs of elements interact directly except x and z .

	x	y	z	u
x	0	1	0	1
y	1	0	1	1
z	0	1	0	1
u	1	1	1	0

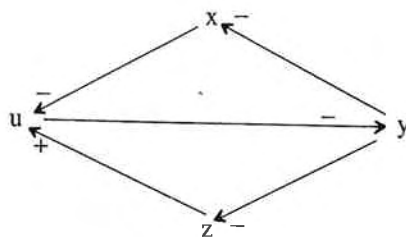
Let us now add the *orientation* of each interaction to the graph:



The adjacency matrix becomes:

Action of \rightarrow on \downarrow	x	y	z	u
x	0	1	0	0
y	0	0	0	1
z	0	1	0	0
u	1	0	1	0

But these interactions can be positive or negative. Specifying these signs gives us the graph:

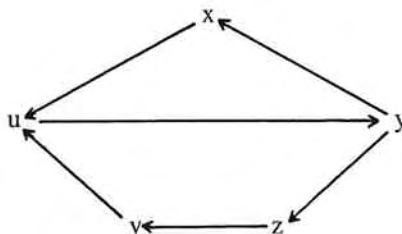


This can be described in the adjacency matrix by replacing the 1s by +s or -s:

Action of \rightarrow on \downarrow	x	y	z	u
x	0	-	0	0
y	0	0	0	-
z	0	-	0	0
u	-	0	+	0

Finally, element u is acted on by two elements; one must thus mention whether the connection is AND or OR. This cannot be shown as such in the adjacency matrix. According to whether we are optimistic or pessimistic, we can say that the matrix is so general that it covers both possibilities or that it is unable to distinguish between them. This point is discussed in Chapter 7.

The differences between adjacency matrices and graphs of interactions can be reconciled. As pointed out by Van Ham,⁴ the two ways in which our graphs differ from classically oriented graphs vanish if, instead of AND, OR, and NOT, one uses a single connective, NAND or NOR (cf. Chapter 2, Section II.C). If one introduces the auxiliary element $v = \bar{z}$, the graph can be converted into



in which all interactions are NAND. Note that in this representation there are neither signs for the arrows nor additional information about the connections; *the graph has become a conventional (unsigned) oriented graph*. There are, as above, two feedback circuits, one with three elements and one with four elements. Here the adjacency matrix has only 1s and 0s and it is not ambiguous since all interactions are NAND:

Action of \rightarrow on \downarrow	x	y	z	u	v
x	0	1	0	0	0
y	0	0	0	1	0
z	0	1	0	0	0
u	1	0	0	0	1
v	0	0	1	0	0

The advantages of this representation proposed by Van Ham are obvious: the graphs of interactions become bona fide oriented graphs and all the background of graph theory can be called when required. This representation should therefore be used for most theoretical developments. However, in routine work we prefer to keep the original type of graph since, with them, each vertex corresponds to a physical element of the system (there are no vertices introduced for formal reasons, like v above) and the adjacency matrix is essentially homologous to the Jacobian matrix of the differential description (cf. Chapter 6).

As described in Chapter 7, when a variable acts at more than one point (as does variable y in our example) we take into account that the threshold concentrations for these actions may be different. For example, in the matrix

$$\begin{bmatrix} 0 & -2 & 0 & 0 \\ 0 & 0 & 0 & -1 \\ 0 & -1 & 0 & 0 \\ -1 & 0 & +1 & 0 \end{bmatrix}$$

the "2" means that the action of variable y on function X requires a higher concentration than its action on function Z .

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2. Thomas, R., Ed., *Kinetic Logic, A Boolean Approach to the Analysis of Complex Regulatory Systems*, Springer Verlag, Berlin, 1979, 29.
3. Thomas, R. and Van Ham, P., Analyse formelle de circuits de régulation génétique: le contrôle de l'immunité chez les bactériophages lambdoïdes, *Biochimie*, 56, 1529, 1974.
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Chapter 4

KINETIC LOGIC II: THE CONDITIONS DETERMINING THE CHOICES AMONG PATHWAYS

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I. INTRODUCTION

In the preceding chapter, we proceeded from the logical structure of a system (described by the graph of interactions) to its possible patterns of behavior (described by the graph of sequences of states). Typically, the successive steps are

1. Verbal description
2. Logical structure (graph of interactions)
3. Logical relations
4. State table
5. Pathways and final states (graph of sequences of states)

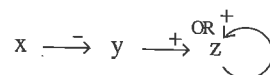
So far, the only simplifying assumption has consisted of treating each interaction as a step function. The state table and the graph of sequences of states follow automatically without further assumptions, except perhaps that when two or more variables are committed to change their value, we do not explicitly consider the possibility that more than one commutation could take place simultaneously. In fact, we do not *exclude* such a possibility; we simply consider it so unlikely that we only show the single commutations explicitly.

Let us now turn to the next problem: *how can we evaluate the conditions that lead a system to follow one pathway rather than another?*

If nothing is said about the length of the time delays, the whole graph of sequences of states remains open; if on the contrary, a well-defined value is ascribed to each time delay, the system will follow a well-defined pathway. We have already touched on this problem in the second example of Chapter 3, and in the present chapter we will consider the question more deeply. However, before this, it is essential to point out that in so doing we would reason as if all the cells of a population followed the same fate. This is, of course, not the case. Even in a homogeneous population of cells, a given delay will have slightly different values from cell to cell; this accounts for the fact that the population can give several responses. As briefly mentioned in Chapter 20 (application to bacteriophage λ), this stochastic aspect of processes can be taken into account. What one can do is ascribe to each time delay an average value and a distribution, and use a computer simulation of many cells, each with one set of delay values picked at random within the distribution.⁴ In this chapter, we will not use the stochastic approach, but rather, will study how the values of the delays — provisionally treated as well defined — influence the decisions. We will first take a slightly more elaborate example than before and show how we currently treat such cases in the “naïve” logical description. In the following section, we treat the stability of logical cycles in the same terms. Only afterwards (in Section IV of this chapter) will we analyze the simplifying assumptions implicitly involved in the “rules of the game” chosen. However, we would like to point out here that a rigorous analysis of the conditions determining the choices of pathways would be a formidable problem and that in practice one *must* use simplifying assumptions at this level. The principal reason for separating these aspects of kinetic logic — behavior patterns and choice of pathway — into separate chapters is that our analysis of the second part (conditions determining the choices) involves an additional degree of idealization.

II. A THREE-VARIABLE EXAMPLE

Let us consider a three-element system¹ consisting of products \underline{x} , \underline{y} , and \underline{z} , coded by genes \underline{X} , \underline{Y} , and \underline{Z} , respectively, which are regulated as follows: gene \underline{X} is expressed constitutively (i.e., not regulated), gene \underline{Y} is expressed only in the absence of product \underline{x} , and gene \underline{Z} is expressed provided product \underline{y} or product \underline{z} is present. The graph of interactions is given by:



and the logical relations are

$$X = 1$$

$$Y = \bar{x}$$

$$Z = y + z$$

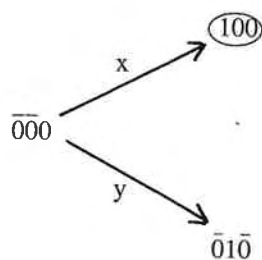
From these the state table is readily constructed:

x	y	z	X	Y	Z
$\bar{0}$	$\bar{0}$	0	1	1	0
$\bar{0}$	$\bar{0}$	1	1	1	1
$\bar{0}$	1	1	1	1	1
$\bar{0}$	1	$\bar{0}$	1	1	1
1	$\bar{1}$	$\bar{0}$	1	0	1
1	$\bar{1}$	1	1	0	1
1	0	1	1	0	1
1	0	0	1	0	0

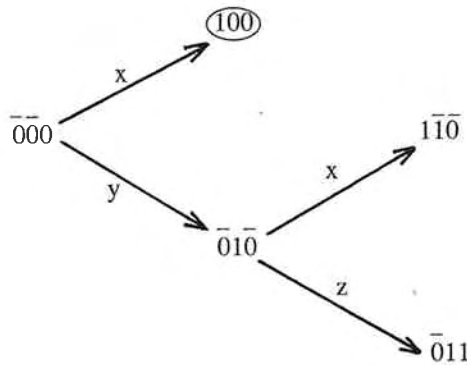
It is immediately apparent that this system has two stable states: (101) (products x and z present, y absent) and (100) (only product x present).

Let us now work out the possible sequences of states, starting from a "virgin" initial state $\bar{0}\bar{0}\bar{0}$. (This situation occurs, for example, after viral infection of a cell which initially contains no viral products). Gene X is turned on immediately after infection and, sooner or later, product x will be and remain present. Gene Y will be expressed until product x appears, switching off gene Y and resulting ultimately in the disappearance of product y . Gene Z will be turned on only if product y (1) reaches its critical threshold (i.e., "appears") before product x has switched gene Y off and (2) remains present long enough.

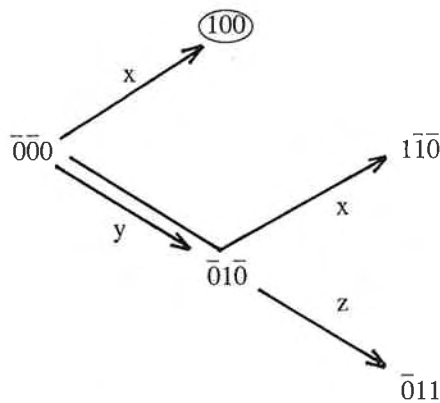
Diagrammatically, from state $\bar{0}\bar{0}\bar{0}$ the system can go to (100) or $\bar{0}1\bar{0}$:



Which path is followed will depend on the relative values of the time delays t_x and t_y . If $t_x < t_y$, the pathway will be $\bar{0}\bar{0}\bar{0} \longrightarrow \textcircled{100}$, whereas if $t_x > t_y$, it will be $\bar{0}\bar{0}\bar{0} \longrightarrow \bar{0}\bar{1}\bar{0}$. In the first case, the system has reached a stable state and will remain there. In the second case, there are again two choices, states $1\bar{1}\bar{0}$ and $\bar{0}\bar{1}\bar{1}$:



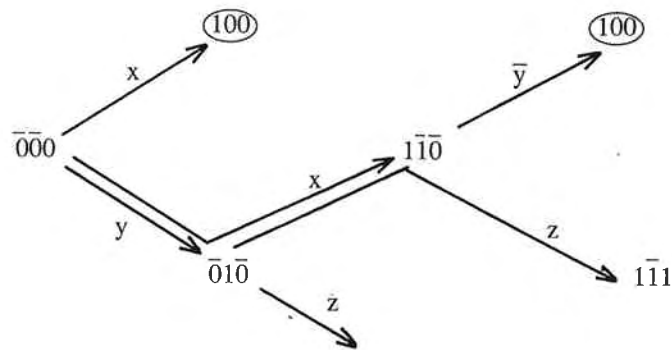
At first, one might expect that the second decision will simply depend on the relative values of t_x and t_z . However, the order to produce z was only given when the system reached state $\bar{0}\bar{1}\bar{0}$, whereas the order to produce x had already been given one step earlier (the order is present at state $\bar{0}\bar{0}\bar{0}$). This can be diagrammed as follows:



From this representation, it is clear that the decision as to which pathway to follow from state $\bar{0}\bar{1}\bar{0}$ will depend on the relative values of t_x and $t_y + t_z$: if $t_x < t_y + t_z$, the decision will be $\bar{0}\bar{1}\bar{0} \rightarrow 1\bar{1}\bar{0}$, whereas if $t_x > t_y + t_z$, it will be $\bar{0}\bar{1}\bar{0} \rightarrow \bar{0}\bar{1}\bar{1}$.

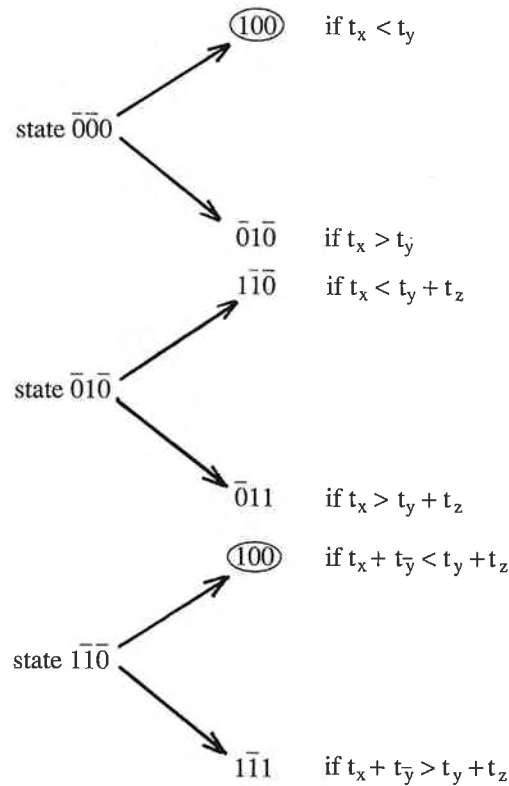
From state $\bar{0}\bar{1}\bar{1}$, there is only one possibility: $\bar{0}\bar{1}\bar{1} \xrightarrow{x} 1\bar{1}\bar{1} \xrightarrow{\bar{y}} \textcircled{101}$. Note, however, that the order to produce x was already present two states earlier.

From state $1\bar{1}\bar{0}$ there is a third decision to be made, again between two possible pathways. Let us use the same representation as above:



It is clear from the diagram that from $1\bar{1}0$ the system will go to $\bar{1}00$ or to $\bar{1}11$ according to whether $t_x + t_{\bar{y}} < t_y + t_z$ or $t_x + t_{\bar{y}} > t_y + t_z$, respectively.

To summarize, there are three decisions:



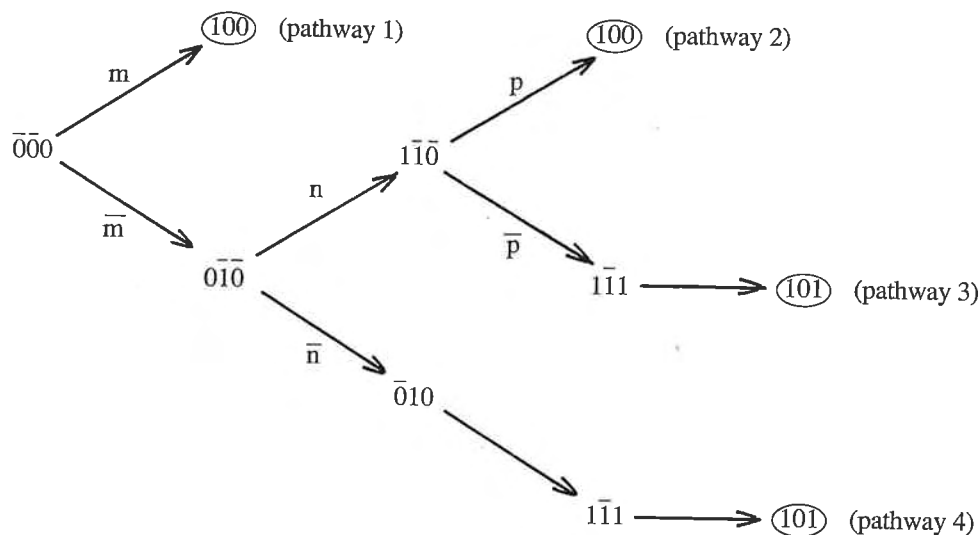
This list provides us with an expression of the qualitative constraints that determine the choices between the pathways. In fact, these constraints on the time delays can be expressed in a more concise and elegant way. For this, it is convenient to associate a logical variable with each of these inequalities:¹

Let $m = 1$ if $t_x < t_y$

$m = 0$ if $t_x > t_y$

(Again, we do not explicitly treat the marginal case $t_x = t_y$.)

Similarly, we associate variable n with the inequality $t_x < t_y + t_z$ and p with the inequality $t_x + t_{\bar{y}} < t_y + t_z$. One can now draw a graph in which the choices are described in terms of these variables:



The conditions for following the different pathways can be read directly from this graph:

1. For pathway 1, the condition is m .
2. For pathway 2, the condition is $\bar{m}np$.
3. For pathway 3, the condition is $\bar{m}n\bar{p}$.
4. For pathway 4, the condition is $\bar{m}\bar{n}$.

This can be tabulated as follows. Let $F1$ be a 4-valued function that takes the value 1 if the conditions lead to pathway 1, etc.

$F1$	00	01	11	10	mn
0	4	3	1	1	
1	4	2	1	1	
p					

Instead of considering each individual pathway, we can also reason that pathways 1 and 2 lead to the final state 100 and pathways 3 and 4 to the final state 101 . Let $F2$ be a function that takes the value 1 when the conditions lead to state 101 and the value 0 when they lead to state 100 . Then $F2 = \bar{m}n\bar{p} + \bar{m}\bar{n}$ (the sum of the conditions for pathways 3 and 4) and $\bar{F2} = m + \bar{m}np$ (the sum of the conditions for pathways 1 and 2). From the table:

$F2$	00	01	11	10	mn
0	1	1	0	0	
1	1	0	0	0	
p					

one can see that this simplifies to:

$$\overline{F2} = m + np \text{ and } F2 = \bar{m}(\bar{n} + \bar{p})$$

These expressions can be further simplified using the following reasoning. If $t_x < t_y$ (condition m), one necessarily has $t_x < t_y + t_z$ (condition n) because time delays are positive; thus, $m \longrightarrow n$, which means that the situation $m\bar{n}$ cannot occur. Similarly, if $t_x + t_y < t_z$ (condition p), one necessarily has $t_x < t_y + t_z$ and so $p \longrightarrow n$, which means that the situation $p\bar{n}$ cannot occur. Consequently, in the state table of a function of m , n , and p , we place dashes in the squares corresponding to $m\bar{n}$ and to $\bar{n}p$ to indicate that the function can be assigned arbitrary values there:

$F'1$	00	01	11	10	mn
0	4	3	1	—	
1	—	2	1	—	
p					

Applying the method described in Chapter 2, Section IV, it can be seen that the condition for:

pathway 1 is m

pathway 2 is $\bar{m}p$

pathway 3 is $\bar{m}n\bar{p}$

pathway 4 is \bar{n}

The table of $F2$, defining the conditions leading to state $\textcircled{000}$ or to state $\textcircled{101}$, can be similarly simplified:

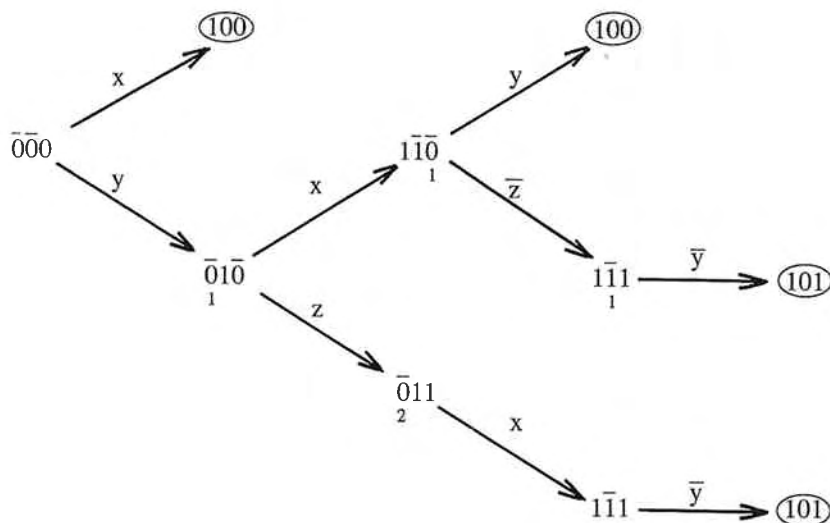
$F'2$	00	01	11	10	mn
0	1	1	0	—	
1	—	0	0	—	
p					

Thus, the condition for reaching state $\textcircled{100}$ ($F2 = 0$) is $m + p$ and the condition for reaching state $\textcircled{101}$ ($F2 = 1$) is $\bar{m}\bar{p}$. In terms of the time delays of our system, we can say that, from a virgin initial state $\bar{0}\bar{0}\bar{0}$, the system will end up in the stable state $\textcircled{100}$ if either or both

of the conditions $t_x < t_y$ or $t_x + t_{\bar{y}} < t_x + t_z$ are fulfilled, and it will go to (101) if both conditions $t_x > t_y$ and $t_x + t_{\bar{y}} > t_x + t_z$ are fulfilled. Note that the expressions of the conditions leading to (100) and (101) are complementary in the present case. This need not always be so, however; there can be an overlap in the states that never occur.

When an order to change a particular variable was given 1, 2, ..., n steps earlier, we note a subscript 1, 2, ..., n . For example, in state $\bar{0}1\bar{0}$, the order to switch on variable x was given one state earlier, so we write $\bar{0}1\bar{0}_1$; in state $\bar{0}11$, the order to switch on variable x was given two states earlier, so we write $\bar{0}11_2$.

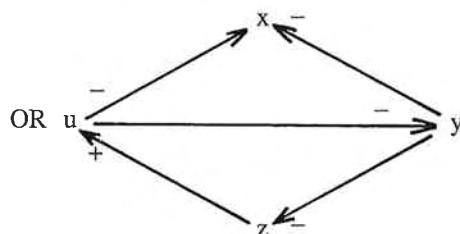
In this notation, the graph of sequences of states becomes:



We would like to draw attention to the two occurrences of the logical state $\bar{1}\bar{1}1$. In one case, the order to switch y from 1 to 0 was already present in the preceding state ($\bar{1}\bar{1}\bar{0}$) and we write $\bar{1}\bar{1}1_1$. In the other case, the same order has just been given and we write $\bar{1}\bar{1}1$. Thus, these two logical states are different and must be treated so.

III. STABILITY ANALYSIS OF LOGICAL CYCLES

Consider a small network comprising four genes \underline{X} , \underline{Y} , \underline{Z} , and \underline{U} such that genes \underline{X} and \underline{Z} are repressed by product \underline{y} , gene \underline{Y} is repressed by product \underline{u} , and gene \underline{U} is repressed by product \underline{x} unless product \underline{z} is present. This system², already used in Chapter 3, Section IV.C, has two conjugated feedback loops, one positive and one negative:



The naïve logical description is

$$X = \bar{y}$$

$$Y = \bar{u}$$

$$Z = \bar{y}$$

$$U = \bar{x} + z$$

and the state table is

x	y	z	u	X	Y	Z	U
$\bar{0}$	$\bar{0}$	$\bar{0}$	$\bar{0}$	1	1	1	1
$\bar{0}$	0	$\bar{0}$	1	1	0	1	1
$\bar{0}$	0	1	1	1	0	1	1
$\bar{0}$	$\bar{0}$	1	$\bar{0}$	1	1	1	1
0	1	$\bar{1}$	$\bar{0}$	0	1	0	1
0	$\bar{1}$	$\bar{1}$	1	0	0	0	1
0	$\bar{1}$	0	1	0	0	0	1
0	1	0	$\bar{0}$	0	1	0	1
$\bar{1}$	1	0	0	0	1	0	0
$\bar{1}$	$\bar{1}$	0	$\bar{1}$	0	0	0	0
$\bar{1}$	$\bar{1}$	$\bar{1}$	1	0	0	0	1
$\bar{1}$	1	$\bar{1}$	$\bar{0}$	0	1	0	1
1	$\bar{0}$	1	$\bar{0}$	1	1	1	1
1	0	1	1	1	0	1	1
1	0	$\bar{0}$	$\bar{1}$	1	0	1	0
1	$\bar{0}$	$\bar{0}$	0	1	1	1	0

Note that variable y affects the expression of genes X and Z . There is no reason to suppose that the thresholds for these interactions will be the same; thus, in a more elaborate treatment, we should consider *two* thresholds and consequently *three* logical values for vari-

able y (see the last section of Chapter 3). This type of treatment is developed in detail in Chapter 7.

From the state table, one can derive a complex graph of sequences of states, a representative part of which can be found in Leclercq and Thomas² and in Appendix 4, Section II. We will not describe the whole graph here. Suffice it to say that the system can follow a number of cyclic pathways which have four states in common and which are, in fact, different modes of the same cyclic attractor. The other attractor is the stable state (1011) .

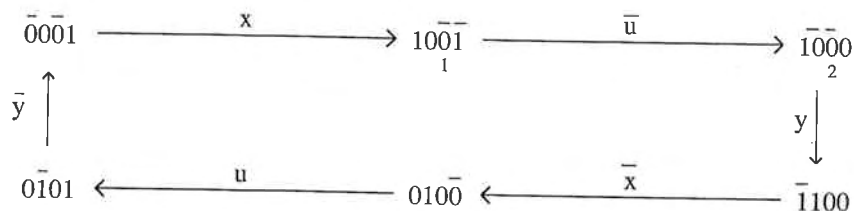
As an illustration, we will consider in more detail two sequences originating from the "virgin" state $\bar{0}\bar{0}\bar{0}\bar{0}$, arbitrarily chosen as initial state:

$$\begin{array}{ccccccccccc} \bar{0}\bar{0}\bar{0}\bar{0} & \xrightarrow{x} & 1\bar{0}\bar{0}\bar{0} & \xrightarrow{y} & \bar{1}100 & \xrightarrow{\bar{x}} & 010\bar{0} & \xrightarrow{u} & 0\bar{1}01 & \xrightarrow{\bar{y}} & \bar{0}\bar{0}\bar{0}1 & \xrightarrow{x} & \\ & & & & & & & & & & & & \\ & & & & 10\bar{0}\bar{1} & \xrightarrow{\bar{u}} & 1\bar{0}\bar{0}\bar{0} & \xrightarrow{y} & \bar{1}100 & \xrightarrow{\bar{x}} & \dots & & \end{array}$$

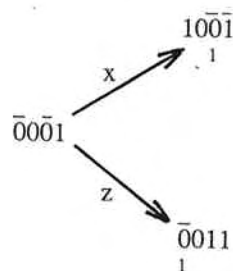
At first, one might think that the sequence between the two occurrences of state $1\bar{0}\bar{0}\bar{0}$ forms a cycle. However, at the first occurrence, functions Y and Z have both been on since the preceding state ($\bar{0}\bar{0}\bar{0}\bar{0}$), so the detailed description of the state is $1\bar{0}\bar{0}\bar{0}_{11}$. At the second occurrence, function Y was switched on precisely when state $1\bar{0}\bar{0}\bar{0}$ was reached, whereas function Z was already on two steps earlier; the detailed description of the state is thus $1\bar{0}\bar{0}\bar{0}_2$. As these two states are different, the sequence between them is *not* a cycle:

$$\begin{array}{ccccccccccc} \bar{0}\bar{0}\bar{0}\bar{0} & \xrightarrow{x} & 1\bar{0}\bar{0}\bar{0}_{11} & \xrightarrow{y} & \bar{1}100 & \xrightarrow{\bar{x}} & 010\bar{0} & \xrightarrow{u} & 0\bar{1}01 & \xrightarrow{\bar{y}} & \bar{0}\bar{0}\bar{0}1 & \xrightarrow{x} & \\ & & & & & & & & & & & & \\ & & & & 10\bar{0}\bar{1}_1 & \xrightarrow{\bar{u}} & 1\bar{0}\bar{0}\bar{0}_2 & \xrightarrow{y} & \bar{1}100 & \xrightarrow{\bar{x}} & \dots & & \end{array}$$

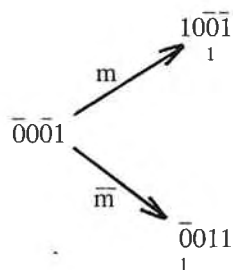
Consider now state $\bar{1}100$, which follows state $1\bar{0}\bar{0}\bar{0}$. In this case, the two occurrences are identical since, in both situations, function X was switched off exactly when the state itself was reached. Thus, the sequence between the two occurrences of $\bar{1}100$ is a cycle:



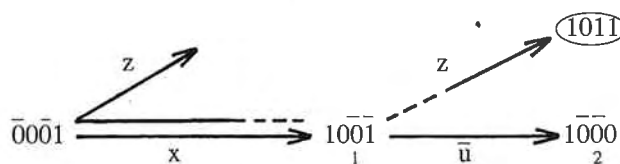
States $\bar{1}100$, $010\bar{0}$, and $0\bar{1}01$ have only one possible follower each. State $\bar{0}\bar{0}\bar{0}1$ has two possible followers:



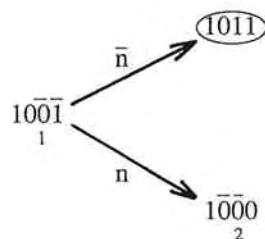
Let $m = 1$ if $t_x < t_z$ and $m = 0$ if $t_x > t_z$. The conditions for the choice are thus:



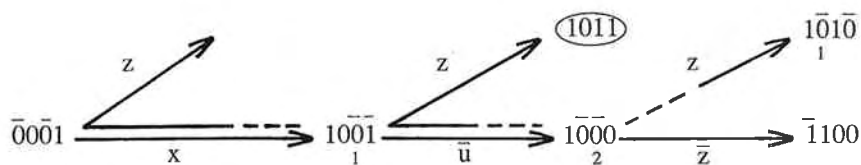
For state $100\bar{1}$, the situation is slightly more complicated:



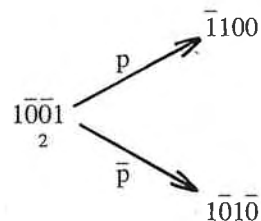
Let $n = 1$ if $t_x + t_{\bar{u}} < t_z$ and $n = 0$ if $t_x + t_{\bar{u}} > t_z$. The conditions for the choice are



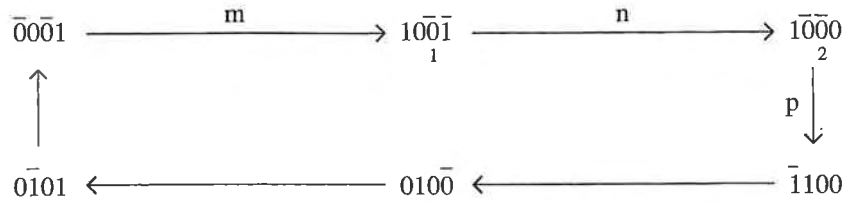
For state $100\bar{0}$, the situation is



Let $p = 1$ if $t_x + t_{\bar{u}} + t_y < t_z$ and $p = 0$ if $t_x + t_{\bar{u}} + t_y > t_z$. The conditions for the choice are

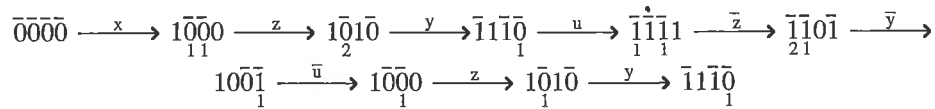


The conditions for following the cycle are

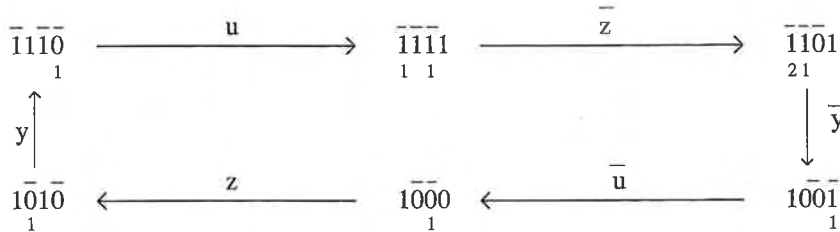


Once reached, the cycle will be followed if mnp . These conditions can be simplified, however: if $t_z < t_x$, then $t_z < t_x + t_u$, which, in turn, implies $t_z < t_x + t_u + t_y$. Thus, $\bar{m} \rightarrow \bar{n} \rightarrow \bar{p}$ or $p \rightarrow n \rightarrow m$. The condition for remaining in the cycle thus reduces to p , that is, $t_x + t_u + t_y < t_z$.

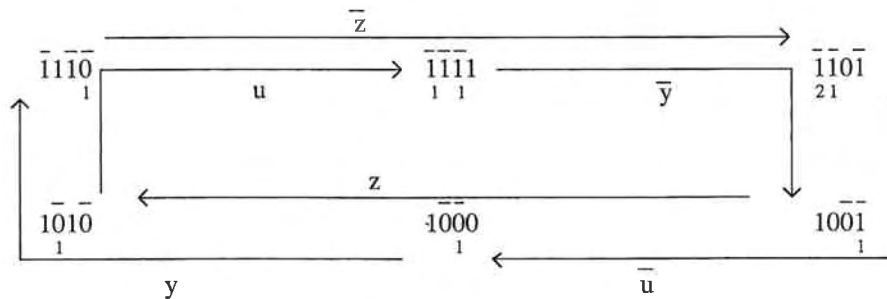
We consider, now, another sequence originating from 0000 :



Since the two occurrences of 1110 carry the same indices, we treat the intervening sequence as a cycle:



Here, every state has two or three potential followers. So there is a choice at each step and we can expect six sets of conditions for remaining in the cycle. However, the time delays form two overlapping circuits, which creates a dilemma.



Ignoring the additional constraints involving t_x , the condition for remaining in the cycle at states 1001 , 1010 , and 1111 is

$$t_y + t_z + t_u < t_y + t_z + t_u,$$

whereas the condition for remaining in the cycle at states $1\bar{0}\bar{0}0$, $\bar{1}1\bar{1}0$, and $\bar{1}10\bar{1}$ is

$$t_y + t_z + t_u < t_{\bar{y}} + t_z + t_u$$

These two inequalities are obviously incompatible. If the first obtains, the system will ultimately leave the cycle at $1\bar{0}\bar{0}0$, $\bar{1}1\bar{1}0$, or $\bar{1}10\bar{1}$, whereas if the second obtains, the system will escape at $100\bar{1}$, $1\bar{0}1\bar{0}$, or $\bar{1}\bar{1}\bar{1}1$.

One possible way out would be to set $t_{\bar{y}} + t_z + t_u = t_y + t_z + t_u$ as a necessary (although not sufficient) condition to stay in the cycle. So far, we have chosen to ignore the marginal situation in which two time delays (or sums of delays) are exactly equal. Indeed, biological time delays are not mathematical constants, but fluctuate slightly (see Section I above). This attitude, which previously led us not to consider the simultaneous commutation of several variables, seems particularly justified here: even though there exist values of the time delays such that the system, once in the cycle, would remain there, the slightest change in value or fluctuation of a single time delay would eject the system from the cycle. In other words, the cycle in question is intrinsically *unstable*. Unlike the first cycle considered, it is not an attractor of the system.

The situation is nicely illustrated by a simulation of this system on "Delphine", the logic machine developed by Van Ham.³ Using the method described in this chapter, one can find values of the time delays such that the system will enter the cycle and remain in it indefinitely. If at this point any single delay is slightly modified, in every case the system ultimately leaves the unstable cycle.

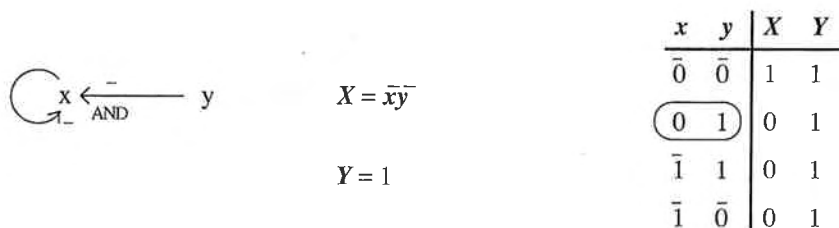
This system will be reexamined in Appendix 4. The point we wish to make here is simply that there are two types of cycle. In the first, the conditions for the system to remain in it are a set of *inequalities* between time delays (or sums of time delays), whereas in the second, one (or more) of the conditions is an *equality*. We call the first type *stable* cycles because one can find values of the time delays such that, once in the cycle, the system remains there even if the values of the delays fluctuate slightly (there are special cases of cycles which, once reached, are followed indefinitely, whatever the values of the time delays; cf. Chapter 3, Section II, first example). The second type we call *unstable* cycles because, although there are values of the time delays for which the system will enter and remain in the cycle, the slightest change in value of certain delays will irreversibly remove the system from the cycle.

In practice, we recognize unstable cycles by the occurrence of overlapping circuits of time delays that result in the presence of subscripts for each state of the cycle.

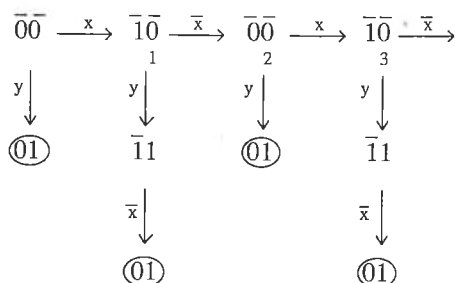
In fact, the situation can be described with three different degrees of precision. A very rough description mentions only the Boolean states, a more refined description in addition assigns subscripts to variables, and a fully precise description keeps track of the remainder of each time delay at the moment a logical state is reached. The remainders are easily obtained with a computer program by subtracting from each time delay the time during which an unexecuted order has been on. Thus, strictly speaking, two states described by the same Boolean vector with the same subscripts are really identical only if they begin with identical remainders; the same subscripts are a necessary but not sufficient condition for identity. The term "cycle" is therefore really appropriate only for circuits in which the remainders are the same from turn to turn. For unstable cycles, this is the case in the marginal situation which would keep the system indefinitely in the circuit; otherwise, the values of the remainders will shift at each turn.

A fortiori, when a Boolean state occurs repeatedly with different indices, we do not have a cycle. Suppose gene \underline{X} is repressed by either product \underline{x} or \underline{y} ; in other words, the gene works

only if the products are both absent. The graph of interactions, logical relations, and state table are



and the sequence of states is



One must beware not to take sequences like $\bar{0}\bar{0} \xrightarrow{1} \bar{1}\bar{0} \xrightarrow{2} \bar{0}\bar{0} \xrightarrow{3} \bar{1}\bar{0} \rightarrow$ for cycles since no Boolean state occurs twice with the same indices. In this case, the gene \underline{Y} is on permanently, so its product *must* eventually appear.

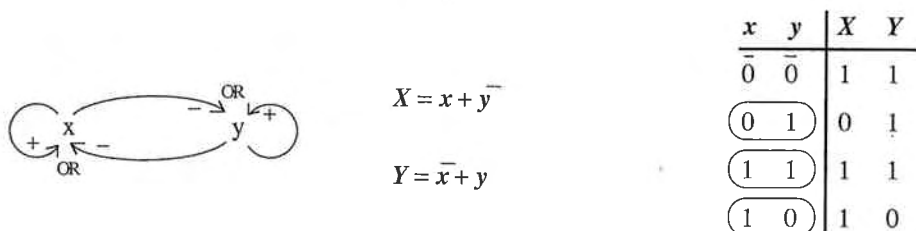
A final warning may be in order here. We have seen above how to determine the conditions under which the system will remain in a cycle. In real situations, however, the initial state is usually not in the cycle. It is therefore important to analyze which initial states *can* lead the system to the cycle, to determine the constraints on the time delays which will effectively allow the system to enter the cycle, and then to verify that these constraints are compatible with the conditions for remaining in the cycle. It may happen, for example, that certain initial states will lead to a state in the cycle only under conditions which will force the system to leave the cycle at another state. The conditions for entering the cycle from a given initial state are determined as in Section II.

IV. TIME DELAYS: THE SIMPLIFYING ASSUMPTIONS UNDERLYING THE ANALYSIS

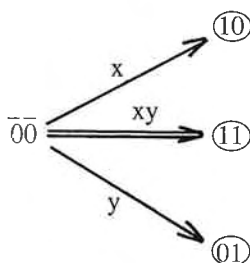
The introduction of specific on and off delays for each element of a system is certainly one of the strong points of our method, making it a useful approximation of biological reality. These time delays are still idealizations, however. In our "naïve" logical description, the principal assumptions concerning the delays, discussed below, are that (A1) different delays (or sums of delays) are never exactly equal, (B1) two constant delays, "on" and "off" are sufficient to describe each element of the system, and (C1) the delays are independent of the past states (or history) of the system.

A. ARE DIFFERENT TIME DELAYS ALWAYS DIFFERENT?

The sequence of states a system will follow depends on the relative values of certain time delays, as explained in Section II above. We usually assume that two delays (or sums of delays) are never exactly equal and therefore that two variables will not change their values at precisely the same instant, although, in fact, we do not exclude the possibility. If this rule were applied rigidly, it could occasionally lead to the loss of interesting pathways. Consider, for example, the system described by the following graph of interactions, equations, and state table:



It has three stable states, one of which can be reached from outside only by a double commutation:



In such cases, one might wish to admit this possibility; it would in no way change the formalism. In our generalized logical treatment (described in Chapter 7), the term concerning each interaction is ascribed a specific weight. Depending on the values of these parameters, all three stable states of the present example can be reached from state 00.

It is perhaps worth pointing out that a synchronous description, which postulates simultaneous commutations, would be much worse than our naïve description: it would admit only the state 11 as successor to 00, eliminating all possibility of choice and thus all possibility of reaching states 10 or 01 from outside.

Since biological delays are never absolutely invariable mathematical constants, but have a distribution around an average value, it is completely unrealistic to imagine that a system could be maintained permanently in an unstable cycle which, as seen above (Section III), would require exact equality of certain sums of delays. This same intrinsic fluctuation of time delays can also lead to situations in which a system seems to make random decisions at branch points where the delays determining which pathway to follow are not too different, with overlapping distributions. To account for this, one can give each time delay an average value and a distribution (see Chapter 20, Section II).

B. ARE TWO TIME DELAYS PER ELEMENT SUFFICIENT?

In synchronous descriptions, as used by virtually everyone else, not only are the "on" and "off" delays equal for each function, but all delays for all functions are equal. This type of model is clearly inadequate to describe many, if not most, biological systems.

So far, we have assigned two time delays, "on" and "off", to each pair of internal variables/functions in a system. The delays represent the time required between the order to turn on and the effective appearance of a substance (commutation from 0 to 1) or between the order to turn off and the effective disappearance (commutation from 1 to 0). However, it must be realized that a given variable change can result from various orders. For example, in the system described in Section III, we have $U = \bar{x} + z$. Function U can be switched on as a result of the disappearance of \bar{x} , as in $\bar{1}100 \xrightarrow{\bar{x}} 010\bar{0}$, or as a result of the appearance of z , as in $1\bar{0}\bar{0}0 \xrightarrow{z} 1\bar{0}1\bar{0}$. It is perfectly conceivable that the "on" delay of variable u be different, according to whether the function has been switched on by the loss of \bar{x} or by the appearance of z . This would be the case for example, if gene \underline{U} could be expressed from two promoters of unequal efficiencies, one repressed by the negative regulator \bar{x} and one requiring the positive regulator z .

Similarly, if $X = y\bar{z}$, the *disappearance* of \bar{x} can be due to the disappearance of y or to the appearance of z . This would be the case if y were a positive regulator required for expression of gene \underline{X} and z a protease which destroys the \bar{x} product. Thus, if y disappears, \bar{x} will disappear because it is no longer synthesized, and if z appears, \bar{x} will disappear because it is destroyed. In many cases, the second process will be much faster than the first, and one must thus postulate two very different "off" delays for the same variable.

In addition, when we use multilevel variables, we have to consider transitions from 0 to 1, from 1 to 2, etc. and vice versa. Of course, it will usually take different times to go from level 0 to 1 or from level 1 to 2. In this case, we need two "on" (and two "off") delays for the same variable.

The above considerations should alert the reader to the fact that our description, although closer to reality than many others, has implicit assumptions which, in certain cases, can affect the analysis. They should be borne in mind when formalizing a system.

C. ARE TIME DELAYS INDEPENDENT OF THE HISTORY OF THE SYSTEM?

When an internal function and its associated variable have the same value, the situation is stable. If the function changes its value, we consider this an order to change the value of its variable, which will be executed after a specific time delay. If the function returns to its initial value before the time delay has elapsed, we reason as though the original order had been canceled, leaving no trace.

This assumption has the merit of its enormous simplicity. However, it should be used with some caution. For example, when a gene \underline{A} is switched on, the concentration of its product \underline{a} will soon start to increase, although it will not reach its effective concentration until a time t_a later. If the gene is turned off at an earlier time, there will be a subthreshold concentration of \underline{a} . If gene \underline{A} is switched on again before this \underline{a} has decayed, it is clear that the "on" delay will be shortened. Even though product \underline{a} is "absent" in terms of its biological activity, the subthreshold concentration will give the system a head start toward the threshold. This kind of situation, which in practice is rather infrequent, can be handled by using variables with more than two values, according to the general logical treatment described in Chapter 7.

A large variety of interesting systems can be analyzed with the "naïve" logical method without violating any of the above assumptions, and the general logical treatment covers many of the exceptions.

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Chapter 5

INDUCTIVE USE OF KINETIC LOGIC

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I. INTRODUCTION

So far, our concern has been to describe the possible patterns of behavior of systems endowed with a given logical structure: the number and quality of the final states, the pathways to reach them from a given initial state, and the conditions for following one or another pathway. This is an *analytic* or *deductive* approach.

It is tempting to try to use kinetic logic in reverse and ask: given a behavioral pattern, what are the simplest interactions among elements of the system that would permit or impose it? This is a *synthetic* or *inductive* approach.^{1,2} It is generally accepted that, whereas the deductive process, from models or hypotheses to their predictions, is rational, the inductive process, from facts to models which account for them, is essentially intuitive. We describe here a method for making part of the inductive process, from facts to models more rational. We obviously cannot give a formula for proceeding straight from the facts to the right explanation; indeed, a set of experimental observations is usually consistent with many distinct models. We want to find (1) the simplest models that *permit* the observed behavior as well as (2) those which *impose* it, (3) the interactions or logical constraints *common to all* models, i.e., the absolute requirements permitting or imposing the behavior in question, and (4) the simplest *modifications of a preexisting model* to make it compatible with the facts.

The experimental scientist who has had some experience formulating models from facts will notice that we by no means replace the need for biological intuition. A major part of model building is identifying the relevant elements of the system under study, and often considerable insight is required to recognize the role of some apparently insignificant substance or to surmise the existence of a hidden regulatory product. A model in which an essential element is missing will obviously never be right, even if it can formally account for the behavior under study. The addition of irrelevant elements to the description, although formally less dangerous, can in practice obscure the analysis and, in the worst cases, create numerous incorrect models. The methods described here offer no help in these first steps of model construction, which remain the biologist's prerogative.

II. A TWO-ELEMENT EXAMPLE

Our general procedure will be as follows: starting from a set of experimental observations for which we are seeking models, we construct a *partial state table* incorporating the given behavior. We then use Karnaugh maps to find the simplest logical relations compatible with the table, and from these we draw the corresponding graphs of interactions. This is just the reverse of our normal procedure (cf. Chapter 4, Section I).

A concrete example will make the process clear. Suppose we have two genes, \underline{X} and \underline{Y} , whose products interact in such a way that *either or both* can be present stably, but there is no stable state with both products absent. We reason that states 01, 11, and 10 must be stable states, i.e., the vectors \underline{XY} and \underline{xy} are equal, whereas the state 00 is not stable. This gives the incomplete state table:

xy	XY
00	$\overline{00}$
①①	01
①①	11
①①	10

in which $\overline{00}$ in the first line indicates that when xy is 00, the vector XY cannot be 00; it can be anything else: 01, 11, or 10. In this very simple case, there are thus three acceptable models. Their state tables are

xy	XY
00	01
①①	01
①①	11
①①	10

xy	XY
00	10
①①	01
①①	11
①①	10

xy	XY
00	11
①①	01
①①	11
①①	10

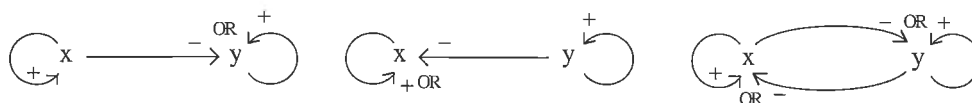
The corresponding logical relations are

$$\begin{cases} X = x \\ Y = \bar{x} + y \end{cases}$$

$$\begin{cases} X = x + \bar{y} \\ Y = y \end{cases}$$

$$\begin{cases} X = x + \bar{y} \\ Y = \bar{x} + y \end{cases}$$

and the respective graphs of interactions are



Once these formal logical circuits have been found, they can in turn be subjected to further analysis. If, for example, the third graph is taken as a model for a differential system, using the methods described in Chapter 6, one finds three stable states corresponding to the Boolean situations 01, 11, and 10 and four additional unstable steady states. In all three models, at least one variable appears in both logical equations. In such situations, there is no *a priori* reason to assume that the threshold values will be the same for the separate interactions. Consequently, it is advisable to refine the analysis by using logical variables with more than two values, as described in Chapter 7.

III. OSCILLATING BEHAVIOR

Our second example is from work on neuron networks by Kling and Székely³ and by Friesen and Stent.⁴ We will see in Part III (Chapter 21) exactly how interactions between neurons can be described in terms of logical variables and functions. For the moment, suffice it to say that the dynamic state of a neuron network can be described by a phase diagram that uses bars of appropriate length to indicate which neurons are on and which are off at any

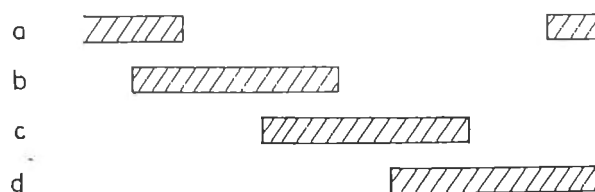
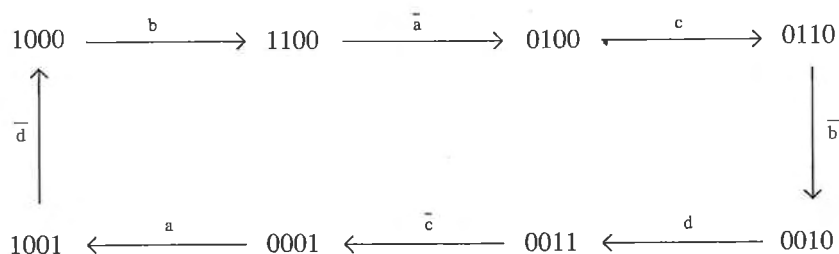


FIGURE 1. Phase diagram of a group of neurons. The abscissa represents time. The neurons a, b, c, and d emit impulses during the hatched periods.

time (Figure 1). This phase diagram is readily translated into a sequence of logical states in which the neurons fire sequentially:



Let us first look for those interactions that *impose* this behavior. In other words, we wish to find models in which the system is forced to follow the cycle indefinitely once one of the above states is reached. This amounts to saying that for each of the eight states in the cycle, the only possible follower is the next state in the cycle; there are no constraints on the other eight states. This gives us the following incomplete state table:

<i>abcd</i>	<i>ABCD</i>
0000	----
0001	1001
0011	0001
0010	0011
0110	0010
0111	----
0101	----
0100	0110
1100	0100
1101	----
1111	----
1110	----
1010	----
1011	----
1001	1000
1000	1100

The table contains 32 unspecified values, indicated by dashes; there are thus 2^{32} ($\approx 4 \times 10^9$) different ways to construct connections that would impose this behavior! To find the simplest circuits, we first rewrite the state table in the form of Karnaugh maps, with a different subtable for each function:

<i>A</i>	00	01	11	10	<i>ab</i>
00	—	0	0	1	
01	1	—	—	1	
11	0	—	—	—	
10	0	0	—	—	
<i>cd</i>					

<i>B</i>	00	01	11	10	<i>ab</i>
00	—	1	1	1	
01	0	—	—	0	
11	0	—	—	—	
10	0	0	—	—	
<i>cd</i>					

<i>C</i>	00	01	11	10	<i>ab</i>
00	—	1	0	0	
01	0	—	—	0	
11	0	—	—	—	
10	1	1	—	—	
<i>cd</i>					

<i>D</i>	00	01	11	10	<i>ab</i>
00	—	0	0	0	
01	1	—	—	0	
11	1	—	—	—	
10	1	0	—	—	
<i>cd</i>					

Reasoning as in Chapter 2, Section III, we choose the most compact logical expression for each function. In the present case, the simplest combination, as shown in the diagram, is

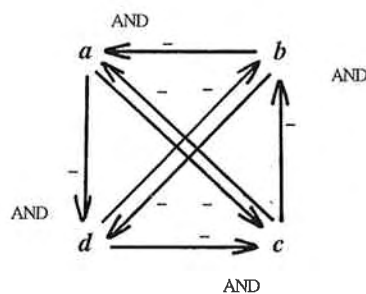
$$A = \bar{b}\bar{c}$$

$$B = \bar{c}\bar{d}$$

$$C = \bar{d}\bar{a}$$

$$D = \bar{a}\bar{b}$$

This corresponds to the logical structure:



It is easy to check that this network (which is the one Friesen and Stent started from) indeed imposes the sequence in question.

In the logical circuit just derived, each element regulates two others. In general, whenever an element acts at more than one point, the thresholds for the different interactions may be different. To handle such situations, we use logical variables with more than two values. This will be discussed in detail in Chapter 7. At this point, we merely wish to warn the reader that the behavior of a system can change when variables are given more than two values. It is thus important to check that the desired properties, in this case the stable cycle, remain unaffected.

We have just seen how to determine the logical conditions that *impose* a particular pattern of behavior. Suppose we now relax this requirement and look for logical circuits which simply *permit* the behavior. We reason as follows. Consider the state 1000. Instead of saying, as above, that the next state *must* be 1100 (and writing 1000/1100), we now say that the next state *may* be 1100, but there may be other transitions as well. To indicate this in the state table, we write 1000/-1- -. This guarantees that 1100 is a possible follower of 1000, but does not specify whether there are others. An extreme case would be $\overline{1000}/0111$, in which the system has a choice among four possible transitions, one of which is 1100. The incomplete state table now becomes:

<i>abcd</i>	<i>ABCD</i>
0000	----
0001	1---
0011	--0-
0010	---1
0110	-0--
0111	----
0101	----
0100	--1-
1100	0---
1101	----
1111	----
1110	----
1010	----
1011	----
1001	---0
1000	-1--

Here, there are 56 dashes. There are thus 2^{56} ($\approx 7 \times 10^{16}$) different ways of connecting neurons *A*, *B*, *C*, and *D* that permit the desired cycle. Rewriting the table in the form of Karnaugh maps gives:

<i>A</i>	00	01	11	10	<i>ab</i>
00	—	—	0	—	
01	1	—	—	—	
11	—	—	—	—	
10	—	—	—	—	
<i>cd</i>					

<i>C</i>	00	01	11	10	<i>ab</i>
00	—	1	—	—	
01	—	—	—	—	
11	0	—	—	—	
10	—	—	—	—	
<i>cd</i>					

<i>B</i>	00	01	11	10	<i>ab</i>
00	—	—	—	1	
01	—	—	—	—	
11	—	—	—	—	
10	—	0	—	—	
<i>cd</i>	—	0			

<i>D</i>	00	01	11	10	<i>ab</i>
00	—	—	—	—	
01	—	—	—	0	
11	—	—	—	—	
10	1	—	—	—	
<i>cd</i>					

The simplest functions fitting these constraints are

$$A = \bar{a}, \text{ or } A = \bar{b}, \text{ or } A = d,$$

$$B = \bar{b}, \text{ or } B = \bar{c}, \text{ or } B = a,$$

$$C = \bar{c}, \text{ or } C = \bar{d}, \text{ or } C = b,$$

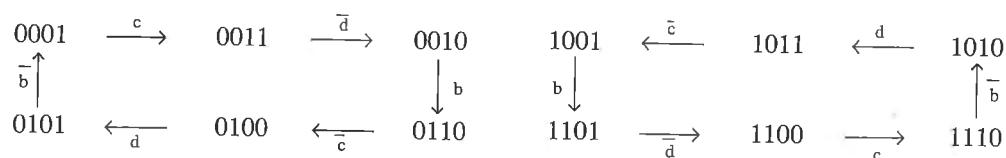
$$D = \bar{d}, \text{ or } D = \bar{a}, \text{ or } D = c.$$

Any combination of these functions will be formally compatible with the desired cycle. In some cases, however, the cycle turns out to be unstable. This is the case, for example, for $A = d$, $B = a$, $C = b$, and $D = c$, as the reader can readily verify. Each state in the cycle has a subscript index, and the time delays form two overlapping circuits, as in the example treated in Chapter 4, Section IV.

If we wish to find logical solutions in which our cycle is a stable attractor, the easiest way is to *impose* stability, rather than testing the stability of the cycle in randomly chosen combinations of functions. To do this, we choose one state of the cycle and impose the following state as the *only* possible next state. (This is what we did above for all eight states of the cycle when we wanted to ensure that the system, once in the cycle, could never leave it.) The simplest logical functions compatible with the new state table are derived from Karnaugh maps, as usual. The cycle will now have a state with only one possible follower. This follower will therefore carry no indices, and there will be values of the time delays for which the cycle will be a stable attractor. In the present case, one such combination, derived by imposing state 1100 as the only possible follower of 1000, is $A = \bar{b}$, $B = a$, $C = b$, and $D = c$.

IV. A CHOICE BETWEEN TWO LIMIT CYCLES

Suppose we wish to find a logical circuit which, according to the initial state, will permit a choice between two stable cycles running in opposite directions. Using our logical formalism, we will represent the cycles as follows:



The order of commutations in the two cycles is $\bar{b}c\bar{d}b\bar{c}d$ and $d\bar{c}b\bar{d}c\bar{b}$, respectively; this is what is meant by "running in opposite directions".

We wish to impose these cycles in the sense that once in either cycle, the system cannot leave it. We proceed as in the preceding example, constructing an incomplete state table in which each state in a cycle can be followed only by its successor in the cycle. As before, the states that occur in neither cycle are not specified. The state table is

<i>abcd</i>	<i>ABCD</i>
0000	----
0001	0011
0011	0010
0010	0110
0110	0100
0111	----
0101	0001
0100	0101
1100	1110
1101	1100
1111	----
1110	1010
1010	1011
1011	1001
1001	1101
1000	----

The corresponding Karnaugh maps are

<i>A</i>	00	01	11	10	<i>ab</i>								
00	—	0	1	—	<div><table><tr><td>1</td><td>—</td></tr><tr><td>1</td><td>1</td></tr><tr><td>—</td><td>1</td></tr><tr><td>1</td><td>1</td></tr></table></div>	1	—	1	1	—	1	1	1
1	—												
1	1												
—	1												
1	1												
01	0	0	1	1									
11	0	—	—	1									
10	0	0	1	1									
<i>cd</i>													

<i>B</i>	00	01	11	10	<i>ab</i>								
00	—	1	1	—	<div><div></div><table><tr><td>—</td><td>1</td></tr><tr><td>1</td><td>1</td></tr><tr><td>—</td><td>0</td></tr><tr><td>1</td><td>0</td></tr></table></div>	—	1	1	1	—	0	1	0
—	1												
1	1												
—	0												
1	0												
01	0	0	1	1									
11	0	—	—	0									
10	1	1	0	0									
<i>cd</i>													

<i>C</i>	00	01	11	10	<i>ab</i>
00	—	0	1	—	
01	1	0	0	0	
11	1	—	—	0	
10	1	0	1	1	
<i>cd</i>					

<i>D</i>	00	01	11	10	<i>ab</i>
00	—	1	0	—	
01	1	1	0	1	
11	0	—	—	1	
10	0	0	0	1	
<i>cd</i>					

The simplest logical expressions, shown on the above maps, are

$$A = a$$

$$B = \bar{a}c + \bar{a}\bar{d}$$

$$C = \bar{a}d + \bar{a}\bar{b}$$

$$D = a\bar{b} + \bar{a}\bar{c}$$

This logical system behaves as expected: depending on the initial state, the system will go to one or the other cycle and stay there. Starting from these logical equations, we have constructed and analyzed an equivalent differential system⁵ using differential equations as explained in Chapter 6. Again, the system behaved exactly as expected: for appropriate parameter values (which were easy to find), the system would go to one or the other of two limit cycles (Chapter 10), depending on the initial state.

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Chapter 6

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I. INTRODUCTION

A classical description of dynamic systems uses ordinary differential equations. The equations give the *time derivative* dx/dt of each relevant element \underline{x} of a system as a function of the *level* of the elements and, when required, of external conditions. For example, for a chemical system comprising three compounds \underline{x} , \underline{y} , and \underline{z} whose concentrations are represented by x , y , and z , we write:

$$\frac{dx}{dt} = H_x(x, y, z, \dots),$$

$$\frac{dy}{dt} = H_y(x, y, z, \dots),$$

$$\frac{dz}{dt} = H_z(x, y, z, \dots).$$

If the functions H_x , H_y , and H_z are known, then for a given initial state (x_0, y_0, z_0, \dots) , the equations in principle permit one to compute the state of the system at any time t .

II. THE NOTION OF STEADY STATE

This section provides a simple example of the differential description of a synthetic process. It also introduces the fundamental notion of *steady state*. A steady state is defined as a state for which none of the variables change with time (the time derivatives are nil). For instance, a substance produced at a constant rate, but decaying at a rate proportional to its concentration, will not accumulate indefinitely, but rather, its concentration will tend toward a limit steady-state value. *In particular, when a gene is not subjected to any specific control, the concentration of its product nevertheless does not increase uncontrollably, but approaches a limit steady-state value* because the product decays, or diffuses, or is diluted out by the growth of the system. This is reminiscent of the limit speed of a falling object. The object is submitted to a constant force (its weight) that would generate a constant acceleration if things were simple. However, as speed increases, a frictional force directed opposite to the motion develops, and this force increases in proportion to its speed. When the speed is such that the vertical component of the frictional force almost equals gravity, the resultant force is negligible and the body goes on falling, but at a constant speed, called the "limit speed".

Let us first consider the case of a substance \underline{x} which is synthesized from a precursor \underline{a} at a rate proportional to the concentration of \underline{a} , which we consider essentially constant. We imagine our system immersed in a reservoir of \underline{a} sufficiently large that the overall concentration of \underline{a} is not significantly lowered by the reaction under consideration. The rate of synthesis of \underline{x} is thus ka . On the other hand, as for all substances considered here, \underline{x} is subject to spontaneous decay, which is usually assumed to be proportional to the concentration of \underline{x} itself. The time derivative dx/dt , which represents the *net* rate of synthesis of \underline{x} , will thus have a positive term (synthesis) and a negative term (decay):

$$\frac{dx}{dt} = ka - k_-x \tag{1}$$

in which k and k_- are (positive) kinetic constants; a and x , which are concentrations, are also nonnegative.

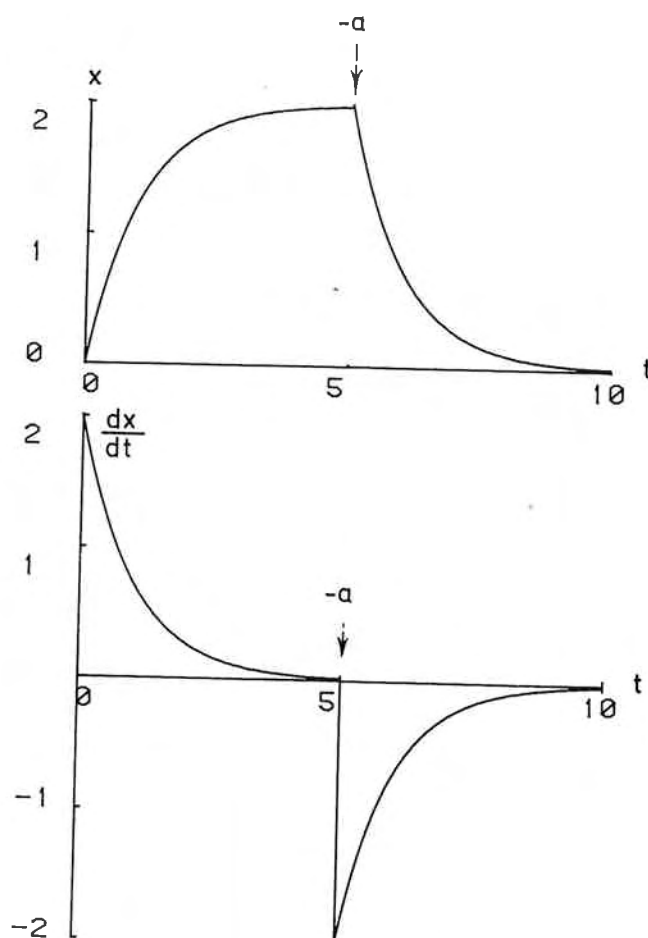


FIGURE 1. The system $\frac{dx}{dt} = ka - k_-x$. Above: plot of x as a function of time. Initially, $x = 0$. At time 0, one switches on x synthesis by adding the precursor a . The arrow indicates that one removes a . Below: plot of $\frac{dx}{dt}$ in the same conditions.

Let us start from an initial state in which $x = 0$ and the process of synthesis is switched on by adding the precursor at concentration a . As the initial value of x is 0, the term of decay is initially nil and $dx/dt = ka$; but as x is produced, the term k_-x will steadily increase and dx/dt will decrease by that much, tending toward 0. Accordingly, the concentration x will increase linearly at first (at rate ka), then more slowly, eventually approaching a steady-state value x^0 (Figure 1).

Since the steady-state value x^0 is defined by $dx/dt = 0$, when $x = x^0$ we have $ka - k_-x^0 = 0$, and the steady-state value is simply

$$x^0 = \frac{ka}{k_-}$$

Thus, when the synthetic process is switched on (here, by providing the system with a required precursor), the new *rate* of accumulation first jumps from 0 to ka , then decreases as

\underline{x} is formed, and approaches 0. The concentration of \underline{x} will first increase almost linearly (with slope ka), then progressively level off, approaching the steady-state value $\frac{ka}{k_-}$. The exact kinetics can be obtained by integrating Equation 1, which gives:

$$x = \frac{ka}{k_-} (1 - e^{-k_-t}).$$

If, starting from x^0 , one now removes \underline{a} , the first term of Equation 1 disappears and $dx/dt = -k_-x$. Initially, $x = x^0 = \frac{ka}{k_-}$; thus $\frac{dx}{dt} = -ka$.

The concentration of \underline{x} thus declines with a rate $-k_-x$ which initially equals $-ka$, but as x decreases, the rate of decay itself declines and approaches 0: the system tends toward another steady-state value, $x^0 = 0$. The variation of x and dx/dt with time is shown in Figure 1.

Let us now consider an "unregulated" gene. "Unregulated" will be used here to qualify a gene not subject to a control specifically addressed to it or to the operon (transcriptional unit) to which it may belong. For constant temperature and flow of precursors, the product of such a gene would be synthesized at a constant rate. However, it will not accumulate and reach intolerable concentrations because gene products are dissipated by degradation, diffusion, growth dilution, or otherwise at rates assumed to be proportional to their own concentration. As in the chemical reaction considered above, if the gene product y is synthesized at a constant rate k and degraded at a rate k_-y , it will not accumulate indefinitely, but, rather, will tend toward a steady-state concentration $y = k/k_-$.

III. NONLINEAR INTERACTIONS

In the simple case described above, the rate of synthesis of \underline{x} was a linear function of the precursor concentration \underline{a} ; we speak of a linear interaction between \underline{a} and \underline{x} . In regulatory processes, most interactions are nonlinear. On the one hand, many regulators have little or no effect below a critical "threshold" concentration. On the other hand, the effect of a regulator usually levels off at sufficient concentrations (boundary concentrations).

This S-shaped type of nonlinearity is called *sigmoid* (from the Greek letter "sigma", Σ). Sigmoid curves are monotonic, with a lower and upper bound, near-zero derivatives for small and large values of the arguments, and a single inflection point. Most authors describe sigmoid curves by the so-called Hill functions. Here, we will symbolize increasing and decreasing Hill functions by kF^+ and kF^- , respectively ($k > 0$), and consider only nonnegative values of x .

We write $F^+(x)$ or, more explicitly, $F^+(x, \theta)$:

$$\begin{aligned} F^+(x, \theta) &= \frac{x^n}{\theta^n + x^n} = \frac{(x/\theta)^n}{1 + (x/\theta)^n} & F^-(x, \theta) &= \frac{\theta^n}{\theta^n + x^n} = \frac{1}{1 + (x/\theta)^n} \\ F^+(x, \theta) &= 0 \text{ for } x = 0 & F^-(x, \theta) &= 1 \text{ for } x = 0 \\ &= 0.5 \text{ for } x = \theta & &= 0.5 \text{ for } x = \theta \\ &\rightarrow 1 \text{ for } x \rightarrow \infty & &\rightarrow 1 \text{ for } x \rightarrow \infty \end{aligned}$$

Note that

$$F^+(x) = 1 - F^-(x)$$

and

$$F^-(x) = 1 - F^+(x)$$

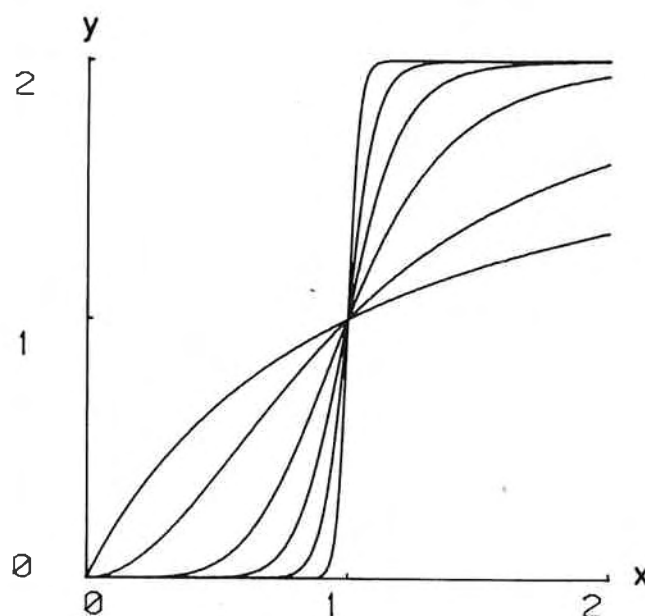


FIGURE 2. Sigmoid functions (more specifically, $y = \frac{2x^n}{1+x^n}$, with increasing values of the Hill number ($n = 1, 2, 5, 10, 20$, and 50).

For $n = 1$, the interaction is of the classical Michaelis-Menten type, and the shape is a branch of a hyperbola; it is bounded, but there is no inflection point. For $n > 1$, Hill curves do have sigmoid shape, and for increasing values of n , the sigmoids become steeper and steeper (and the inflection point closer and closer to $x = \theta$) (Figure 2).

The situation " $n \rightarrow \infty$ " can be approximated by using very high values of n , but it is simpler to use step functions: for $n \rightarrow \infty$, $F^+(x) = 0$ or 1 , according to whether $x < \theta$ or $x > \theta$, and $F^-(x) = 1$ or 0 in the same conditions. It is thus convenient to introduce here the Boolean variable x , and simply write that for $n \rightarrow \infty$:

$$F^+(x) = x$$

and

$$F^-(x) = \bar{x}.$$

When step functions rather than classical sigmoids are used in differential equations, we speak of "piecewise linear" differential equations.^{1,2}

As beautifully shown by Glass & Kauffman,³ many systems have the same qualitative behavior for a wide range of steepness of the sigmoids. Within this range, the exact value of n is thus often of secondary importance. This is why, in situations involving more than one sigmoid function, we often use the same value of n in each.

Strictly speaking, θ is nothing more than the value of x for which F has half of its maximal value. In Michaelis-Menten kinetics ($n = 1$), this concentration of x is called the K_M . However, for sufficient n , one can assume in practice that

$$F^+(x) \approx 0 \text{ for } x < \theta$$

$$F^+(x) \approx 1 \text{ for } x > \theta$$

and

$$F^-(x) \approx 1 \text{ for } x < \theta$$

$$F^-(x) \approx 0 \text{ for } x > \theta$$

It is then quite natural to call θ the "threshold" value of x . We are afraid we will sometimes call θ the threshold even for low values of n , although this is criticizable.

These sigmoid functions can be taken to describe the rate of production of a gene product as a function of x , the concentration of a regulator. If, for example, \underline{x} stimulates its *own* synthesis (a positive feedback loop), we write $dx/dt = kF^+(x) - k_-x$, in which the "synthesis" term $kF^+(x)$ is now nonlinear. For low values of x ($x \ll \theta$), the synthesis term remains near 0, and for high values ($x \gg \theta$), there is no further stimulation due to the bounded character of $F^+(x)$. An important consequence of this is that our system is *bounded*: since $F^+(x) < 1$ for all (nonnegative) x and since k and k_- are positive kinetic constants, it is clear that for $x > k/k_-$ we have $dx/dt < 0$. Thus, whenever x exceeds a certain boundary level (here, k/k_-), the negative derivative ensures that it will move back toward the boundary.

IV. MULTIPLE INTERACTIONS

When the rate of synthesis of an element is influenced by more than one regulator, we usually associate a sigmoid function with each interaction. For example, if efficient synthesis of \underline{z} requires both the "presence" of \underline{x} and the "absence" of \underline{y} (that is, requires that the concentration of \underline{x} be above a threshold θ_x and that that of \underline{y} be below a threshold θ_y), we write:

$$\frac{dz}{dt} = kF^+(x, \theta_x) \cdot F^-(y, \theta_y) - k_-z$$

in which k_-z represents, as usual, the decay of \underline{z} . In this expression, the synthesis term of dz/dt is negligible unless $F^+(x)$ and $F^-(y)$ are both significant, that is, unless x is sufficiently high and y sufficiently low.

If *either* condition is sufficient, we write instead:

$$\frac{dz}{dt} = k_1F^+(x, \theta_x) + k_2F^-(y, \theta_y) - k_-z$$

This expression ensures that the rate of synthesis of \underline{z} will be significant, provided the term in F^+ or F^- (or both) are significant.

Note the parallel between *algebraic multiplication* and the *logical AND* operation, and between *algebraic addition* and the *logical inclusive OR* operation. This provides intuitive justification of the fact that the AND operation is called "logical product" and inclusive OR, "logical sum" (see Chapter 2, Section II).

A given regulatory substance may act in a complex way. Suppose, for instance, that \underline{x} favors the synthesis of \underline{z} at medium concentrations, but inhibits it at a high concentration. In such a case, we write:

$$\frac{dz}{dt} = kF^+(x, \theta_1) \cdot F^-(x, \theta_2) - k_-z$$

with $\theta_2 > \theta_1$.

On the other hand, if \underline{x} favors the synthesis of \underline{z} according to two distinct mechanisms of different efficiencies that require significantly different concentrations, we write:

$$\frac{dz}{dt} = k_1F^+(x, \theta_1) + k_2F^+(x, \theta_2) - k_-z,$$

in which the sum $k_1F^+(x, \theta_1) + k_2F^+(x, \theta_2)$ is a curve with two thresholds (θ_1 and θ_2) and two plateaus (k_1 and $k_1 + k_2$). An example of this type is treated in Chapter 15 (Figure 3).

At this point, we would like to remark that the sum or the product of two Hill functions in x typically has more than one inflection point and more than one plateau. In contrast, the *composition* of Hill functions, as $k_1 F^+[k_2 F^-(x)]$, produces a sigmoid shape, with only one inflection point and one plateau.

In the situations just mentioned, we reason as if there were more than one interaction, even if we are treating a single element that acts at different levels.


Consider a system in which a substance x acts in a complex way on its *own* rate of synthesis:

$$\frac{dx}{dt} = G(x) - k_- x$$

The steady state equation $\frac{dx}{dt} = 0$ is $x = \frac{G(x)}{k_-}$ which, according to the degree (n) of $G(x)$, can have up to n solutions. This means that the system in question, even though it has a single element, can have a number of different steady states. But, whereas the fact that an equation (or system of equations) can have a number of solutions is trivial from a mathematical viewpoint, the fact that a real system may have multiple steady states is by no means trivial. This is because the type of interactions operative in the description of real biological systems has a strictly limited variety of shapes; in fact, they are usually monotonic, as sigmoids are. Instead of using sums or products, or sums of products of sigmoids, one could just as well use a sigmoid whose argument is itself a function of two or more variables ("multivariant sigmoids").

V. A TWO-VARIABLE NEGATIVE LOOP

Let us now consider as an example the two-variable negative loop discussed in Chapter 3


and described by the graph of interactions  The differential equations will be

$$\begin{aligned} \frac{dx}{dt} &= k_1 F_1^-(y) - k_- x = H_x(x, y) \\ \frac{dy}{dt} &= k_2 F_2^+(x) - k_- y = H_y(x, y) \end{aligned} \quad (2)$$

in which F_1^- and F_2^+ are, respectively, decreasing and increasing Hill functions and the k and k_- are positive kinetic constants; x and y , which are concentrations, are nonnegative.

These equations simply mean that dx/dt comprises a positive term (*synthesis*) $k_1 F_1^-(y)$, which equals k_1 for $y = 0$ and approaches 0 for high values of y , and a negative term (*decay*), which is nil for $x = 0$ and increases proportionally to x . Similarly, dy/dt comprises a positive term (*synthesis*) $k_2 F_2^+(x)$, which is nil for $x = 0$ and approaches k_2 for high values of x , and a negative term (*decay*) proportional to y .

In other words, the equations say (in a more precise way) that x is synthesized at a rate that is *inversely* related to the concentration of y and that y is synthesized at a rate which is

directly related to the concentration of \bar{x} . This is exactly what the graph  represents. It is true that the equations say that both \bar{x} and \bar{y} are subject to decay at a rate proportional to their concentration, and one might object that this should be represented in the graph of interactions by negative loops of \bar{x} and \bar{y} on themselves. However, insofar as this decay is not *regulated*, we do not take account of it in either the graph of interactions or the logical equations. In the systems we analyze, we assume that *all* elements are subject to spontaneous decay which is proportional to their concentration. This linear decay may reflect degradation, diffusion, or growth dilution. These nonregulatory types of decay are taken into account in the "off" delays (the time required after a gene is turned off for the corresponding product to drop below its threshold concentration). Like most nonlinear systems, this one cannot be solved analytically, i.e., one cannot find an explicit algebraic expression giving the values of x and y as a function of time (or of each other). Nevertheless, much can be said about the system, as we shall see.

VI. STEADY-STATE EQUATIONS, NULLCLINES, AND STEADY-STATE VALUES

At steady states, all time derivatives are nil:

$$\frac{dx}{dt} = H_x = 0, \quad \frac{dy}{dt} = H_y = 0, \dots$$

In such a situation, the system, in the absence of external change, will remain as it is.

For the system introduced in Section V, the steady state is thus defined by the equations:

$$\begin{aligned} \frac{dx}{dt} &= k_1 F_1^-(y) - k_{-1}x = 0 \\ \frac{dy}{dt} &= k_2 F_2^+(x) - k_{-2}y = 0 \end{aligned} \quad (3)$$

Curves of Equations (3) drawn in the x - y plane (Figure 3) are called "nullclines". Along each of these lines, one time derivative, dx/dt or dy/dt , is nil. Their intersect(s) thus provide us with the point(s) for which both conditions $dx/dt = 0$ AND $dy/dt = 0$ are fulfilled, i.e., the steady-state value(s).

Except for special cases, on one side of a nullcline, the corresponding time derivative is positive; on the other, it is negative. Thus, the nullclines partition the variable space into domains, each characterized by a combination of signs of the time derivatives of each variable (+ +, + -, - -, - +; see Figure 3). For example, in the region labeled + -, dx/dt is positive and dy/dt is negative. Since x is increasing and y is decreasing, the tendency can be symbolized by an arrow directed rightward and downward (\searrow) and similarly, *mutatis mutandis*, for the other regions of the x - y plane.

The steady-state equations can be rewritten:

$$\begin{aligned} x &= \frac{k_1}{k_{-1}} F_1^-(y) = G_1^-(y) \\ y &= \frac{k_2}{k_{-2}} F_2^+(x) = G_2^+(x) \end{aligned} \quad (4)$$

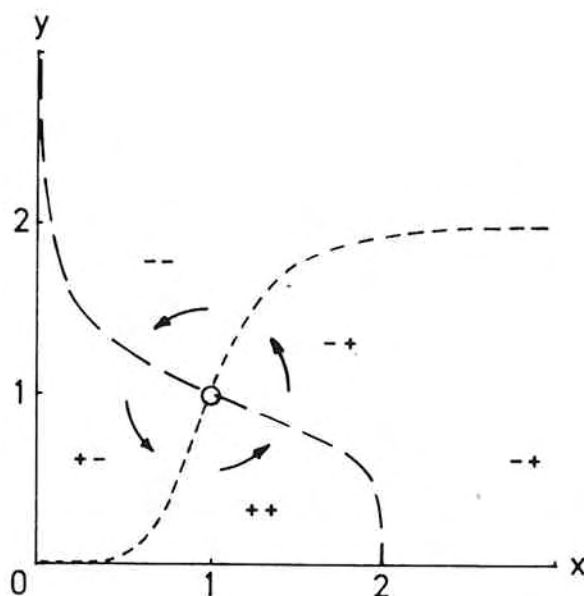


FIGURE 3. The nullclines of System 2. In our particular case:

$$\frac{dx}{dt} = \frac{2}{1+y^5} - x$$

$$\frac{dy}{dt} = \frac{2x^5}{1+x^5} - y$$

The steady-state equations are thus

$$\frac{dx}{dt} = 0 \text{ (---)} : x = \frac{2}{1+y^5}$$

$$\frac{dy}{dt} = 0 \text{ (----)} : y = \frac{2x^5}{1+x^5}$$

The steady state is at the intersect of the two nullclines.

Note that since $y = G_2^+(x)$ and $x = G_1^-(y)$, one can eliminate one variable and write

$$x = G_1^- [G_2^+(x)] = G_3(x),$$

or

$$y = G_2^+ [G_1^-(y)] = G_4(y).$$

(see Richelle⁴). Clearly, $G_3(x)$ and $G_4(y)$ are nonnegative.

This allows one to find the steady-state values(s) as the intersect(s) between the functions x (the bisectrix) and $G_3(x)$ or between y and $G_4(y)$. It is easy to "show", if not to prove, that the composition of Hill functions, like $G_1^- [G_2^+(x)]$, produces a sigmoid shape. Although they are no longer Hill functions and may have a nonzero lower bound, they will be increasing or decreasing sigmoids according to whether there is an *even* or *odd* number of negative sigmoids in the chain.

Thus, in our case $G_1^- (G_2^+(x))$ and $G_2^+ (G_1^-(y))$ are decreasing sigmoids and *there is a single steady state* (Figure 4) because each has exactly one intersect with the bisectrix.

Equations 3 and 4 are the steady-state equations whose solution(s) provide the steady-state value(s) of the system. Note that instead of a system of *differential* equations (2), we now

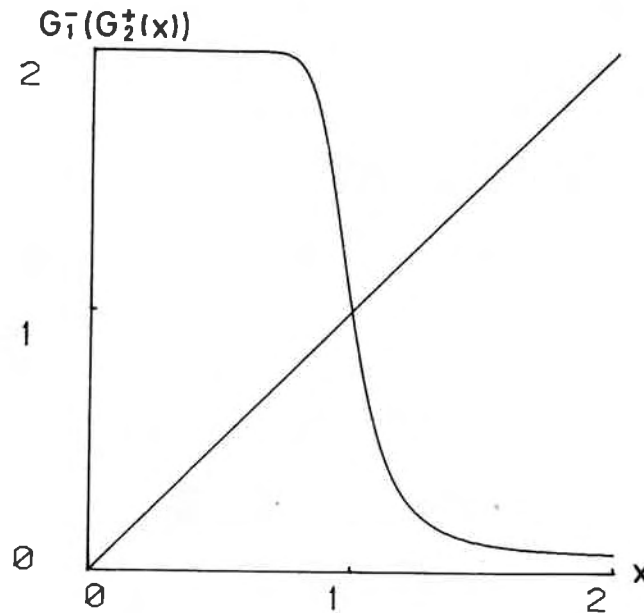


FIGURE 4. The steady-state value x^0 of the system described in Figure 3 can also be found as the intersect of $G_1(G_2^+(x))$ with the bissectrix.

have a simple algebraic system. The differential equations we started from “contained all the information” (as biologists would say) on the *dynamics* of the system, whereas the algebraic equations arrived at have retained only the *static* information concerning the steady-state situation(s).

Yet even the algebraic system usually cannot be solved analytically. As a first step, we have solved it graphically (see Figures 3 and 4). Fortunately, for any given values of the parameters (k_1/k_{-1} , k_2/k_{-2} , θ_x , θ_y , and n), steady states can be *calculated* using an appropriate iterative (numerical) method (see Appendix 1).

This is probably the place to say something about the *dimensions* of these parameters. In the equation

$$\frac{dx}{dt} = k_1 F(x, y, z, \dots) - k_{-1}x$$

(in which F is a combination of sigmoids), $\frac{dx}{dt}$, and consequently each term of the right member of the equation, has the dimensions of x per unit time (xt^{-1}), whatever the nature of x (usually a concentration). As $k_{-1}x$ has the dimensions xt^{-1} , k_{-1} must have the dimension t^{-1} . The term $F(x, y, z, \dots)$ is dimensionless because the variables appear with the same degree in the numerator and in the denominator, so k_1 must have dimensions xt^{-1} .

Thus, the boundary value $\frac{k_1}{k_{-1}}$ (which we will often write K_1) has the dimensions of x , as does θ_1 , which is taken as the threshold level.

When we represent a (say three-dimensional) system in the variable space, we have the points:

$$K_1 = \frac{k_1}{k_{-1}} \text{ and } \theta_x \text{ on the } x \text{ axis,}$$

$$K_2 = \frac{k_2}{k_{-2}} \text{ and } \theta_y \text{ on the } y \text{ axis,}$$

and

$$K_3 = \frac{k_3}{k_{-3}} \text{ and } \theta_z \text{ on the } z \text{ axis.}$$

The parallelepiped built from the origin and points K_1 , K_2 , and K_3 is a box toward which the representative point of the system will always move whenever it lies outside it (see end of Section III).

VII. NATURE OF THE STEADY STATE(S): LINEAR STABILITY ANALYSIS

When a system is *at* steady state, it will remain there since time derivatives of all variables are nil. Suppose, however, that the concentration of one or more components undergoes a small perturbation. Depending on the case, the perturbation may regress and the system tend back toward the steady state it started from or it may amplify and the system eventually proceed to another region of the variable space. These are called *stable* and *unstable steady states*, respectively. As we shall see below, things are not always so simple. For example, an unstable steady state may be attractive along one surface or line, but not elsewhere. In addition, a steady state may be approached (or departed from) *monotonically* or *periodically* (see below).

The nature and stability of steady states can be studied by so-called *linear stability analysis*. In this section, we will just mention the *principle* of the method and give some results of its application to the simple two-variable system already described. More about it can be found in Appendix 3.

The idea is to perturb the system by removing it slightly from its steady state and to check whether the perturbation grows or regresses. *Provided the system is close enough to the steady state x^0 , linear approximations of the differential equations can be used.* It is convenient here to consider the (small) difference ξ between x and x^0 , $\xi = x - x^0$, and similarly for the other variables in multiple variable systems. Close to a steady state, this difference is small enough that the expression of $H(x^0 + \xi)$ can be approximated by the linear term of its Taylor expansion, $\frac{dH(x^0)}{dx} \cdot \xi$ (see Appendix 3), also written

$$\left(\frac{dH}{dx} \right)_{x^0} \cdot \xi.$$

Thus, for a one-variable system, the nonlinear differential equation $\frac{dx}{dt} = H(x)$ is linearized to $\frac{d\xi}{dt} = \omega\xi$, in which ω is simply the value of the derivative $\frac{dH}{dx}$ at the steady state considered:

$$\omega = \left(\frac{dH}{dx} \right)_{x^0}.$$

This value is the slope of $H(x)$ at x^0 (see Figure 1 of Chapter 12).

$\frac{d\xi}{dt} = \omega\xi$ has solutions of the form $\xi = \xi_0 e^{\omega t}$, as can be readily verified by differentiating

the latter expression. Thus, according to whether ω is negative or positive, the perturbation will regress or increase and, accordingly, the steady state will be *stable* or *unstable*.

We mention the following points here without justification (cf. Appendix 3). For a system with two or more variables, the role of the derivative $\frac{dH}{dx}$ is played by the Jacobian matrix, whose elements are the partial derivatives of each function with respect to each variable:

$$\begin{pmatrix} \frac{\partial H_x}{\partial x} & \frac{\partial H_x}{\partial y} \\ \frac{\partial H_y}{\partial x} & \frac{\partial H_y}{\partial y} \end{pmatrix}$$

The stability of a steady state depends on the roots ω of the so-called *characteristic equation*, whose coefficients depend on the terms of the Jacobian matrix, evaluated at the steady state. For an n -variable system, the characteristic equation is of degree n and thus has up to n distinct roots.

Whereas in a one-variable system, a steady state can only be stable (if $\left(\frac{dH}{dx}\right)_{x_0} < 0$) or unstable ($\left(\frac{dH}{dx}\right)_{x_0} > 0$), in a two-variable system, the nature of a steady state depends on two roots: only if *both* are negative will the steady state be stable.

However, this is not the whole story; as is well known, for the quadratic equation $\omega^2 - S\omega + P = 0$, the roots are *real* only if $S^2 - 4P \geq 0$. Otherwise the roots are *complex conjugates*: $\omega_1 = \alpha + i\beta$; $\omega_2 = \alpha - i\beta$, in which α is the *real* part and β is the *imaginary* part. Expressions in $e^{\omega t}$ with complex ω are periodic with time (Euler formula: $e^{i\theta} = \cos \theta + i \sin \theta$). It follows that when the roots of the characteristic equation are complex (i.e., when $S^2 - 4P < 0$), the steady state is approached (if the real part of the roots is negative) or departed from (if the real part of the roots is positive) *periodically*; and we have a *stable* or *unstable focus*, respectively.

It is shown in Chapter 10 that in the case of a two-element negative loop, the (unique) steady state is necessarily stable. For the parameter values used in Figures 3, 4, and 5A, the roots of the characteristic equation are complex conjugates (with a negative real part, which is why the steady state is stable) (see Appendix 3, Example 1). The steady state is thus approached periodically and is a *stable focus*. For other parameter values the characteristic equation has two real negative roots, and the steady state is therefore approached directly and is a *stable node* (see Appendix 3, Example 2, and this chapter, Figure 5B).

In other two-variable systems, we will find other types of steady states: *unstable foci*, or *nodes*, and *saddle points* (which have two real roots, one positive and one negative; see Chapter 12). In systems with more than two variables, we will find steady states with more complex stability properties.

VIII. TRAJECTORIES AND EVOLUTION

The *trajectory* of a system is a curve that depicts its successive states in the variable space. For a one-variable system, the variable space reduces to the x axis (and, when dealing with variables such as concentrations, only the nonnegative part of this axis). For a two-variable system, the variable space is the x - y plane (only its nonnegative or NE quadrant, when dealing with concentrations).

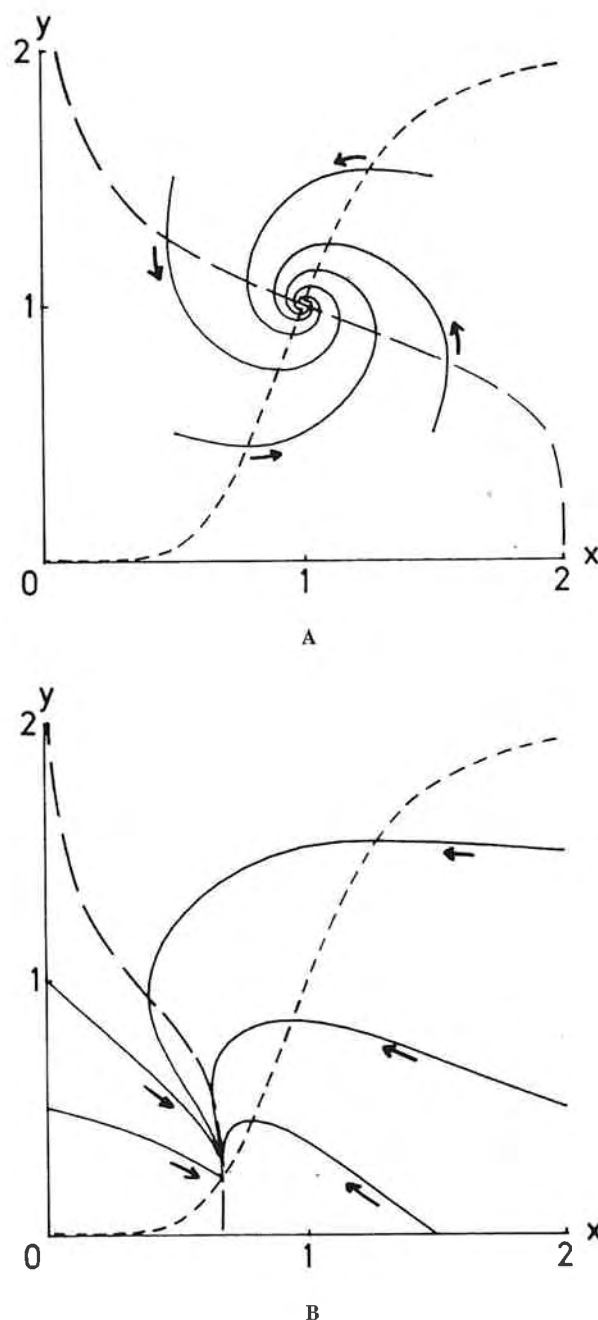


FIGURE 5. Nullclines and trajectories in System 2. (A) Parameter values as in Figure 3 and 4; (B) parameter values as above, except that $k_{-1} = 3$ instead of 1. The trajectories show and the linear stability analysis (Appendix 3, examples 1 and 2) confirms that in (A) the steady state is a stable focus and in (B) it is a stable node.

In the case of nonlinear systems, there is usually no analytic expression for the trajectories. However, for any set of values of the parameters, trajectories can be computed numerically (see Appendix 2).

For the moment, we will simply show trajectories of the two-variable system (2), with two different sets of parameters. A simple look at Figure 5A and 5B shows that the steady

state is approached periodically in A, but not in B. In A, the steady state is a *focus*; in B, it is a *node*, in agreement with the linear stability analysis.

The *evolution* of a system is a description of its state as a function of time (see, for example, Figure 7 in Chapter 10).

IX. SUMMARY

In this chapter, we have seen how dynamic biological control systems can be described with ordinary differential equations, using Hill functions for sigmoid regulatory interactions. The *steady states* of the system — states in which all time derivatives are zero — can be found from these equations. Steady states can be stable or unstable, i.e., for a system at steady state, slight perturbations can regress or amplify. The stability of a given steady state is readily determined by *linear stability analysis*. The differential description of a continuous system can provide the *trajectory* the system will follow through the variable space and its *evolution* in time.

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Chapter 7

GENERALIZED KINETIC LOGIC

E. H. Snoussi, R. Thomas, and R. D'Ari

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I. INTRODUCTION

One drawback of what we now call the naïve logical description is its all-or-none character, with no nuances. The introduction of intermediate levels requires variables and functions with more than two values, but the question of when and how to use them was far from evident. The systematic use of 3-, 4-, ..., n -level variables would simply create better and better imitations of the differential description without resolving the basic problem. A major step forward (obvious in retrospect, like the "oeuf de Colomb"*) came with the realization that there is a natural way of deciding how many levels a given variable should have: whenever an element interacts at more than one place in the graph of interactions, each interaction should have a specific threshold. An element involved in n interactions will therefore have n distinct thresholds, and the corresponding variable and function will require $n + 1$ logical values. (It follows that the different variables of a system need not have the same number of values.) The application of this insight required the development of new methods. We already knew how to handle logical *variables* with more than two values in our analysis (Van Ham¹, Richelle^{2,3}), but until recently we had no fully satisfactory way to assign values to the corresponding logical *functions*.

A primary difficulty with multivalued logical functions is already apparent in the handling of sums (and products) of logical terms. In the naïve description, the same "weight" is attributed to each term and, indeed, to the sum itself. A more refined description should take account of the fact that different terms are usually of different strength and that two or more terms that are individually too weak to carry out a regulatory step effectively may be sufficient when acting together.

To resolve these problems with multivalued logical functions, one of us (E. H. S.) developed generalized logical relations in which the weight of each term is specified by an associated *logical parameter*, and functions as well as variables can take more than two values when required.⁴ This logical formalization includes our naïve logical description as a special case. As we shall see in this chapter and in Chapter 8, it provides a systematic method for extracting all qualitatively different patterns of behavior, not only from discrete logical systems, but also from continuous systems of analogous structure.

This generalized kinetic logic is presented below. We first explain how a discrete scale is ascribed to each variable (Section II) and a characteristic weight to each term (Section III). We then show how to incorporate these new elements in the formalization of a system whose naïve description is an obvious oversimplification (Section IV). Finally, we present the generalized logical description of the same system (Section V).

II. A DISCRETE SCALE FOR EACH VARIABLE

A. GENERALIZED (MULTILEVEL) LOGICAL VARIABLES

Let us consider an element x that acts at various points of a system, with characteristic thresholds $^1\vartheta, ^2\vartheta, ^3\vartheta, \dots, (^1\vartheta < ^2\vartheta < ^3\vartheta \dots)$. We quite naturally associate with this element a multilevel logical variable x , which takes the values:

$$x = 0 \text{ for } x < ^1\vartheta,$$

$$x = 1 \text{ for } ^1\vartheta < x < ^2\vartheta,$$

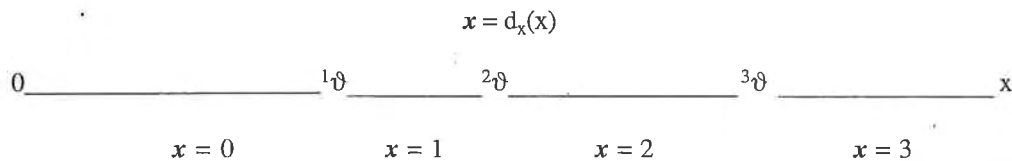
$$x = 2 \text{ for } ^2\vartheta < x < ^3\vartheta,$$

* Reference to Columbus's alleged solution to the problem of how to stand an egg stably on end: crush the end slightly.

$$x = 3 \text{ for } {}^3\vartheta < x < {}^4\vartheta,$$

•
•
•

(We provisionally neglect the marginal case $x = {}^i\vartheta$.) This amounts to applying an operation of discretization to the real variable x according to the scale of thresholds of element \underline{x} . We symbolize this operation d_x and write:

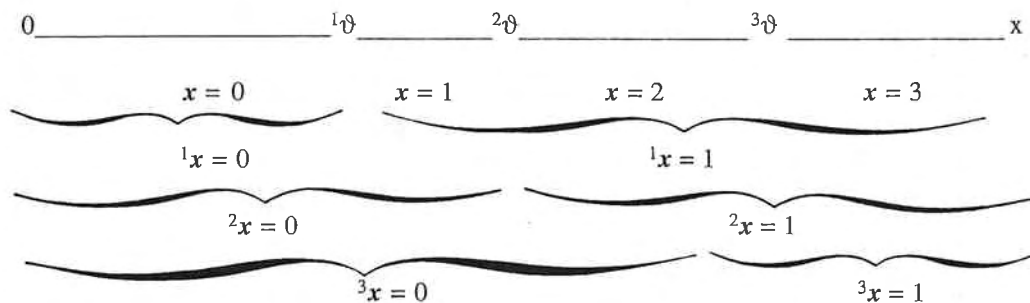


Similarly, the operation d_y is the discretization according to the scale of thresholds of element \underline{y} . These thresholds will, in general, be different from those of \underline{x} both in *number* and in *value*.

B. A COMPLEMENTARY REPRESENTATION: ONE BOOLEAN (BINARY) VARIABLE FOR EACH THRESHOLD

For a given interaction, what interests us is not whether $x = 0, 1, 2, 3, \dots$, but simply whether the level is above or below the threshold for that interaction. For example, if the threshold in question is ${}^2\vartheta$, what we really want to know is whether $x \geq 2$. Therefore, for each multilevel logical variable, we use in parallel a set of Boolean (binary) variables ${}^1x, {}^2x, {}^3x, \dots$, defined as follows:

$$\begin{aligned} {}^1x &= 1 \text{ if } x \geq 1, \text{ otherwise } {}^1x = 0, \\ {}^2x &= 1 \text{ if } x \geq 2, \text{ otherwise } {}^2x = 0, \text{ etc.} \end{aligned}$$



It is important to bear in mind that the multivalued and binary variables are conceptually different and should be clearly distinguished, even when they have the same value. The multivalued variables are used to represent the state of the system, as in the state tables, whereas the binary variables are used in connection with a specific threshold, as in the logical relations. For a system involving a two-level element \underline{x} and a three-level element \underline{y} , for example, we use the three-level variable y together with binary variables 1y and 2y to describe the three-level element \underline{y} ; for the two-level element \underline{x} , although x and 1x have the same value,

three-level element \underline{y} ; for the two-level element \underline{x} , although x and 1x have the same value, we use the former in the entries of the state tables and the latter in the logical relations. Of course, when the description of a system requires *only* two-level variables, we simply use the symbols x, y , etc., since no confusion is possible.

It will be noticed that the binary variables introduced above are not independent; if, for example, in a given state ${}^2x = 1$, we cannot have ${}^1x = 0$, and so forth. In fact, this notation is not very compact: n binary variables, which could potentially describe 2^n states, are used for only $n + 1$ different situations. However, this in no way complicates the analysis, whereas considerable difficulty can arise from the use of symbols whose meaning is not immediately clear.¹

III. A WEIGHT FOR EACH TERM: LOGICAL PARAMETERS

The Boolean logical expression $X = y + z$ means that process X takes place iff product y , product z , or both are present. This classical description does not discriminate among the situations: y alone present, z alone present, and y and z both present. If we wish to do so, we must express the weight of each term and provide a way of evaluating the (multivalued) sum. Let us associate characteristic weights with the terms involving y and z and consider the "pseudo-Boolean" expression $K_1 {}^1y + K_2 {}^1z$, in which K_1 and K_2 are (positive) real numbers and 1y and 1z are Boolean variables. $K_1 {}^1y$ has the value K_1 or 0, according to the value of the Boolean variable 1y , and "+" is the algebraic sum. The values of this expression will be

0 if y and z are both absent,

K_1 if y alone is present,

K_2 if z alone is present,

$K_1 + K_2$ if y and z are both present.

In fact, what we are really interested in is not so much the numerical value of $K_1 {}^1y + K_2 {}^1z$, but its location in the scale of thresholds of variable x , i.e., $d_x(K_1 {}^1y + K_2 {}^1z)$. If variable x has, say, two thresholds, ${}^1\vartheta$ and ${}^2\vartheta$ (${}^1\vartheta < {}^2\vartheta$), the expression $d_x(K_1 {}^1y + K_2 {}^1z)$ will take the values 0, 1, or 2, according to whether:

$$K_1 {}^1y + K_2 {}^1z < {}^1\vartheta,$$

$${}^1\vartheta < K_1 {}^1y + K_2 {}^1z < {}^2\vartheta, \text{ or}$$

$${}^2\vartheta < K_1 {}^1y + K_2 {}^1z.$$

These considerations lead us to write as a multivalued generalization of the logical sum $X = y + z$, the expression:

$$X = d_x(K_1 {}^1y + K_2 {}^1z),$$

in which X is a multivalued logical function.

The usefulness of this definition will appear on examining the results with different values of K_1 and K_2 (Table 1). Let element \underline{x} have two thresholds, ${}^1\vartheta = 1$ and ${}^2\vartheta = 3$; x is thus a three-level logical variable, and the discretization d_x of any real number (other than ${}^1\vartheta$ or ${}^2\vartheta$) will take the value 0, 1, or 2.

TABLE 1

(a) $K_1 = 1.2, K_2 = 1.9$

$K_1^1 y + K_2^1 z$	0	1	y
0	0	1.2	
1	1.9	3.1	
z			

$X = d_x(K_1^1 y + K_2^1 z)$	0	1	y
0	0	1	
1	1	2	
z			

(b) $K_1 = 1.2, K_2 = 1.3$

$K_1^1 y + K_2^1 z$	0	1	y
0	0	1.2	
1	1.3	2.5	
z			

$X = d_x(K_1^1 y + K_2^1 z)$	0	1	y
0	0	1	
1	1	1	
z			

(c) $K_1 = 0.8, K_2 = 0.7$

$K_1^1 y + K_2^1 z$	0	1	y
0	0	0.8	
1	0.7	1.5	
z			

$X = d_x(K_1^1 y + K_2^1 z)$	0	1	y
0	0	0	
1	0	1	
z			

Clearly, the relation $x = d_x(K_1^1 y + K_2^1 z)$ includes several qualitatively different situations according to the values of K_1 and K_2 . Among these situations, that described in (a), with three different values for X , has no counterpart in classical Boolean logical expressions; if y or z is present alone, $x = 1$, but if they are both present, $x = 2$. The situation described in (b) is identical to the classical Boolean sum since $X = 1$ whenever y or z or both are present. And that described in (c) is identical to the classical Boolean product since $X = 1$ only if y and z are both present.

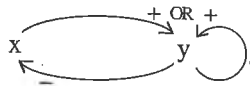
The last point is of special interest. It shows that the generalized logical sum, $X = d_x(K_1^1 y + K_2^1 z)$, includes not only the classical sum, but also the classical product as special cases. The product corresponds to situations in which neither term alone is strong enough to turn X on, but both together are sufficient.

In Chapter 3, Section IV, we mentioned that our "graphs" of interactions not only have to be oriented and signed (+ or -), but also require an indication as to whether multiple interactions are connected with "AND" or "OR". Incidence matrices were considered ambiguous for lack of these indications. The fact that our generalized sum includes the classical sum and product suggests that the apparent ambiguity of incidence matrices (or graphs) without "AND" or "OR" results, in fact, from a greater generality, covering "AND", "OR", and other connections.⁴ (See also King⁵.)

To summarize: the generalized logical sum $X = d_x(K_1^1 y + K_2^1 z)$ provides a rational expression for assigning values to a multilevel logical function. It involves assigning a weight to each term. Depending on the value of these weights, the generalized sum can be identical to the classical sum or the classical product, or it can generate nonclassical situations, including those in which the function value is neither 0 nor 1.

IV. TOWARD A GENERALIZED DESCRIPTION

Consider a two-element system



This circuit comprises a negative loop and a positive loop. Since the two are connected, neither is a simple feedback loop. The naïve logical description is

$$X = \bar{y}$$

$$Y = x + y$$

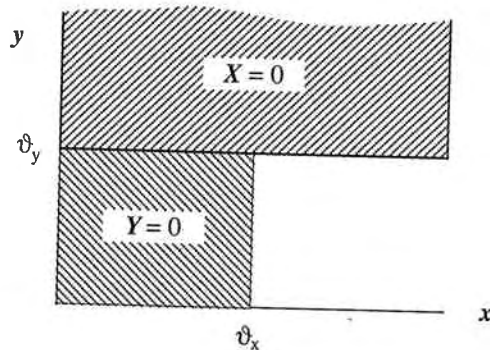
and the state table is

TABLE 2

<i>xy</i>		<i>XY</i>
00		10
⓪1	(a)	01
11		01
10		11

<i>y</i>			
1		⓪1/01 ←	11/01
0		00/10 →	10/11
		0	1 <i>x</i>

The second representation recalls the variable space (the *x*-*y* plane) of the differential system. Thus, according to the naïve logical description, the only pattern of behavior of the system would be $00 \longrightarrow 10 \longrightarrow 11 \longrightarrow \textcircled{01}$. This naïve description can be illustrated in the *x*-*y* plane as follows:



$X = 0$ in the region hatched rightward (hatched), 1 elsewhere, and $Y = 0$ in the region hatched leftward (hatched), 1 elsewhere. This is an alternate form of Table 2(b).

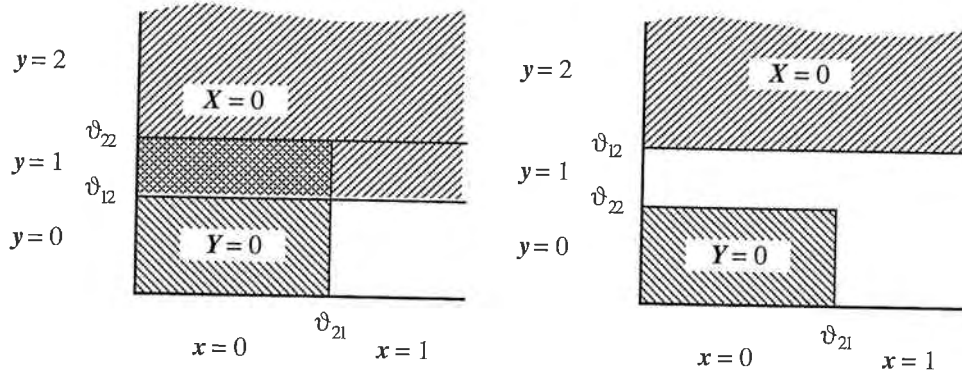
However, y acts both on the synthesis of x (as a repressor) and on its own synthesis (as an activator). One should thus consider two thresholds, which will be denoted ϑ_{12} and ϑ_{22} , respectively; ϑ_{12} concerns the effect on the first variable (x) of the second variable (y), and so forth.

More generally, we will label the threshold ϑ_{ij} , according to the matrix

$$\begin{pmatrix} - & \vartheta_{12} \\ \vartheta_{21} & \vartheta_{22} \end{pmatrix}$$

in which the subscripts recall the location of the terms in the system of logical (or differential) relations; the dash indicates that in the present case there is no effect of x on its own synthesis (i.e., $\vartheta_{11} = 0$).

There are now two qualitatively different situations, according to whether $\vartheta_{12} < \vartheta_{22}$ or $\vartheta_{12} > \vartheta_{22}$.



In the region hatched rightward (▨), $X = 0$, and in the region hatched leftward (▩), $Y = 0$. For the first case (on the left, where $\vartheta_{12} < \vartheta_{22}$), we can say that $X = 0$ unless $y = 0$, and $Y = 0$ unless $x = 1$ or $y = 2$. The question now is: what values should be given to the functions X and Y in other situations? One possibility, developed by Richelle,^{3,4} consists of keeping binary (two-valued) functions despite the multilevel character of the variables. Instead, we will use the generalized logical expressions described in Section III and set:

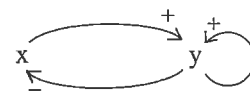
$$\begin{aligned} X &= d_x(K_{12} \overline{^1y}), \\ Y &= d_y(K_{21} \overline{^1x} + K_{22} \overline{^2y}), \end{aligned} \quad (1)$$

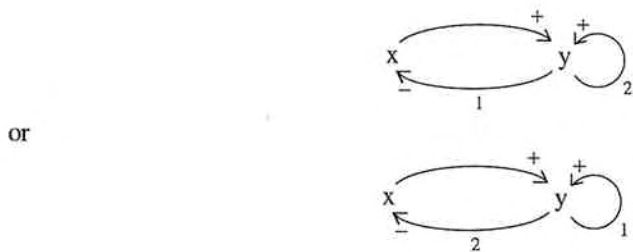
in which the K_{ij} s are real numbers; 1x , 1y , and 2y are Boolean variables associated with the multilevel logical variables x and y ; "+" is the algebraic sum; and d_x and d_y represent discretization according to the scales of x and y , respectively. Similarly, for the second case (on the right, where $\vartheta_{12} > \vartheta_{22}$), the relations are

$$\begin{aligned} X &= d_x(K_{12} \overline{^2y}), \\ Y &= d_y(K_{21} \overline{^1x} + K_{22} \overline{^1y}) \end{aligned} \quad (2)$$

V. GENERALIZED LOGICAL DESCRIPTION

We have now seen the principles underlying the generalized logical description and are ready to carry out the analysis. As an example, we again choose the system

. In view of the above considerations, we ascribe two thresholds to element y : ϑ_{12} concerning its interaction with x ($y \xrightarrow{-} x$), and ϑ_{22} , concerning its interaction with itself ($y \xrightarrow{+} y$). The variable y and function Y will thus have three possible values: 0, 1, and 2. Element x will have a single threshold, ϑ_{21} , corresponding to the interaction $x \xrightarrow{+} y$, so the variable x and function X will be two-valued. According to whether $\vartheta_{12} < \vartheta_{22}$ or $\vartheta_{12} > \vartheta_{22}$, we write the graphs of interactions



respectively. The numbers on the arrows indicate the relative thresholds for the two interactions carried out by y . The logical relations are given in Equations 1 and 2, respectively, or, in a more compact way, by the matrices of interactions:

$$\begin{pmatrix} 0 & -1 \\ 1 & 2 \end{pmatrix}, \begin{pmatrix} 0 & -2 \\ 1 & 1 \end{pmatrix}$$

We shall treat the first case, in which $\vartheta_{12} < \vartheta_{22}$. The relations in Equation(s) 1 give the values of X (0 or 1) and Y (0, 1, or 2) in terms of the Boolean variables 1x , 1y , and 2y that, in turn, are directly determined by the values of x (0 or 1) and y (0, 1, or 2), as described in Section II. The state table is readily constructed from these relations:

x	y	X	Y
0	0	$d_x(K_{12})$	0
0	1	0	0
0	2	0	$d_y(K_{22})$
1	0	$d_x(K_{12})$	$d_y(K_{21})$
1	1	0	$d_y(K_{21})$
1	2	0	$d_y(K_{21} + K_{22})$

To simplify the notation, we set $d_x(K_{12}) = K_{12}$, $d_y(K_{21}) = K_{21}$, $d_y(K_{22}) = K_{22}$, and $d_y(K_{21} + K_{22}) = K_{21+22}$. The integers K_{12} , K_{21} , K_{22} , and K_{21+22} are called "logical parameters". The state table thus becomes:

TABLE 3

$x \ y$		X	Y
0	0	K_{12}	0
0	$\bar{1}$	0	0
0	2	0	K_{22}
1	0	K_{12}	K_{21}
$\bar{1}$	1	0	K_{21}
$\bar{1}$	2	0	K_{21+22}

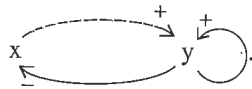
y		
2	$02 / 0 K_{22} \leftarrow \bar{1}2 / 0 K_{21+22}$	
1	$0\bar{1} / 00 \leftarrow \bar{1}1 / 0 K_{21}$	
0	$00 / K_{12} 0$	$10 / K_{12} K_{21}$
	0	1
	x	

Table 3(a) is a classical state table; the representation in (b) again recalls the variable space (x-y plane) of the differential description.

From the definition of the logical parameters, it is clear that K_{12} can take the value 0 or 1 since the scale of x has one threshold (making X a two-valued function), and K_{21} , K_{22} , and K_{21+22} can each take the values 0, 1, or 2 since the scale of y has two thresholds (making Y a three-valued function), with the constraint that K_{21+22} cannot be less than K_{21} or K_{22} . It is easy to show that there are 28 possible sets of values for the logical parameters K_{12} , K_{21} , K_{22} , and K_{21+22} . Each set of values corresponds to a choice of weights for the different terms and will produce a state table specifying a particular pattern of behavior. A number of qualitatively different behavioral patterns will emerge. It can already be seen in Table 3 that certain transitions will be called for independently of the values of the logical parameters; others will appear only for certain sets of values. Now that variables may have more than one value, we use + and - overscripts instead of dashes to indicate when and in which direction a variable is expected to change its value. The state tables for three sets of values are presented in Table 4, using the maximal values for K_{12} (1), K_{22} (2), K_{21+22} (2), and letting K_{21} take the values 0, 1, or 2.

We will discuss the three situations in Table 4 case by case.

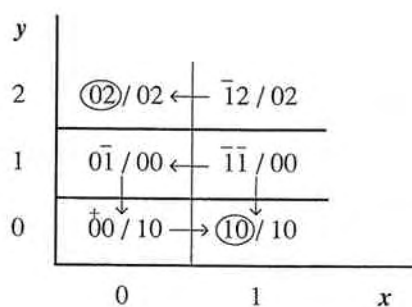
In Case 1, we have $K_{21} = 0$, or $d_y(K_{21}) = 0$, meaning that the term $K_{21} x$ is not effective by itself. This can be represented graphically as follows:



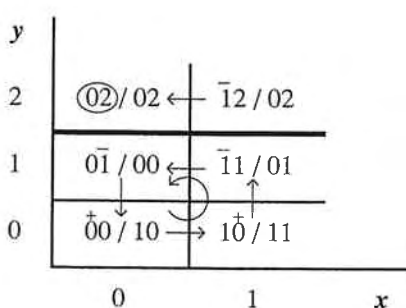
In this circuit, even when synthesis of \underline{x} is fully derepressed (i.e., when \underline{y} is absent), the level of \underline{x} is insufficient by itself to activate the synthesis of \underline{y} . In this situation, it can be seen that there are two separate domains according to whether $y > 1$ or $y \leq 1$, with no transitions passing from one to the other. The corresponding attractors are two stable states, (02) and (10).

TABLE 4

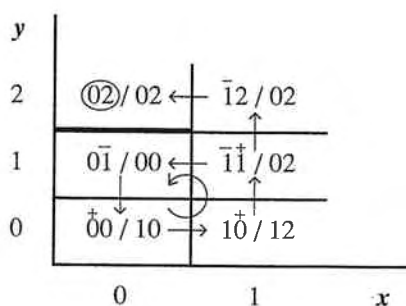
$$1. K_{12} = 1, K_{21} = 0, K_{22} = 2, K_{21+22} = 2$$



$$2. K_{12} = 1, K_{21} = 1, K_{22} = 2, K_{21+22} = 2$$



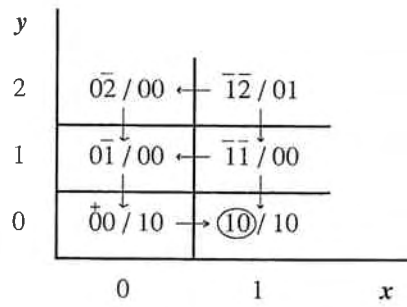
$$3. K_{12} = 1, K_{21} = 2, K_{22} = 2, K_{21+22} = 2$$



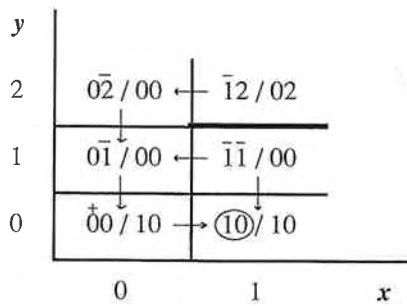
In Case 2 of Table 4, $K_{21} = 1$, so the interaction $x \xrightarrow{+} y$ is effective and the level of \underline{x} attained in state $1\bar{0}$ will be sufficient to stimulate the synthesis of \underline{y} , which will then repress the synthesis of \underline{x} . On the other hand, the concentration of \underline{y} required to repress \underline{x} , ϑ_{12} , is less than that needed to activate its own synthesis, ϑ_{22} , so when \underline{x} synthesis is repressed and \underline{x} disappears, the synthesis of \underline{y} will be switched off and \underline{y} will also disappear. Here again, there are two separate domains according to whether $y = 2$ or $y < 2$. The corresponding attractors are a logical stable state (02) and a logical cycle

Classical product:

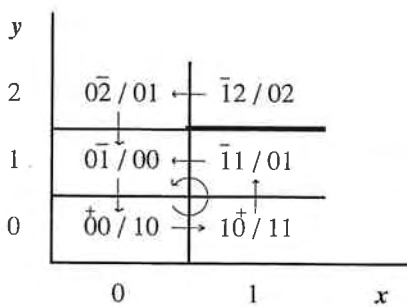
6. $K_{12} = 1, K_{21} = 0, K_{22} = 0, K_{21} + 22 = 1$



7. $K_{12} = 1, K_{21} = 0, K_{22} = 0, K_{21} + 22 = 2$



8. $K_{12} = 1, K_{21} = 1, K_{22} = 1, K_{21} + 22 = 2$



Finally, we will look at the situation when the two thresholds of variable y are not significantly different and show that, even here, the generalized logical description is useful. The analysis is as above, except that variable y now has a single threshold and thus only two levels, 0 and 1. The equations become:

$$X = d_x(K_{12}y)$$

$$X = d_y(K_{21}x + K_{22}y)$$

in which the Boolean variables x and y are written without indices since the system no longer includes any multivalued variables. The single state table is

xy	X	Y
00	K_{12}	0
01	0	K_{22}
11	0	K_{21+22}
10	K_{12}	K_{21}

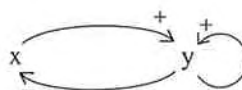
or

y	$x=0$	$x=1$
1	$01 / 0 K_{22}$	$11 / 0 K_{21+22}$
0	$00 / K_{12} 0$	$10 / K_{12} K_{21}$

Let us consider three sets of parameter values:

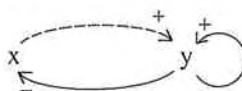
$$9. K_{12} = 1, K_{21} = 1, K_{22} = 1, K_{21+22} = 1$$

y	$x=0$	$x=1$
1	$\textcircled{01} / 01$	$\bar{1}1 / 01$
0	$\bar{0}0 / 10$	$\bar{0}1 / 11$



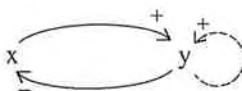
$$10. K_{12} = 1, K_{21} = 0, K_{22} = 1, K_{21+22} = 1$$

y	$x=0$	$x=1$
1	$\textcircled{01} / 01$	$\bar{1}1 / 01$
0	$\bar{0}0 / 10$	$\textcircled{10} / 10$



$$11. K_{12} = 1, K_{21} = 1, K_{22} = 0, K_{21+22} = 1$$

y	$x=0$	$x=1$
1	$0\bar{1} / 00$	$\bar{1}1 / 01$
0	$\bar{0}0 / 10$	$\bar{0}1 / 11$



The dotted lines in the graphs of interactions indicate that the logical value of the corresponding term is weak. When, as here, the terms involve positive interactions, this is equivalent

to considering that the interactions activate synthesis (here, of y) too weakly to bring the concentration up to its threshold value. (In the case of a negative interaction, setting the logical parameter equal to zero would not mean that the *negative interaction* is weak, in which case synthesis would be constitutive, but rather that the *term* itself is negligible, making synthesis always low.)

It can be seen that, whereas the naïve logical description has only a single pattern of behavior, the generalized description has several, even when only Boolean variables are used. Case 9, with a single stable state $\textcircled{01}$, corresponds to the naïve logical description for the system, with the two positive interactions on y connected by "OR". Case 10, on the other hand, has two stable states, and Case 11 exhibits oscillating behavior. The latter situation is of particular interest, as discussed in Chapter 8.

Depending on the values of the logical parameters, the system can display a single stable state with no cyclic pathway, as in the naïve description (Cases 7 and 9), pure multistationarity with two stable states (Cases 1 and 10), pure oscillation with a cyclic pathway but no stable state (Cases 4, 8, and 11), or multistationarity with both a stable state and a cyclic pathway (Cases 2 and 3). In Case 3, the cycle will be followed only for appropriate values of certain time delays.

VI. SUMMARY

In this chapter, we have shown how the naïve logical description can be generalized to accommodate situations in which some variables assume more than two values, without seriously complicating the analysis. Any variable has a "natural" number of biologically relevant levels, determined by the number of elements the product \bar{x} regulates. Each regulatory interaction has a specific threshold, so if \bar{x} regulates n elements, it will have up to n different thresholds and therefore $n + 1$ meaningful levels: $0, 1, \dots, n$.

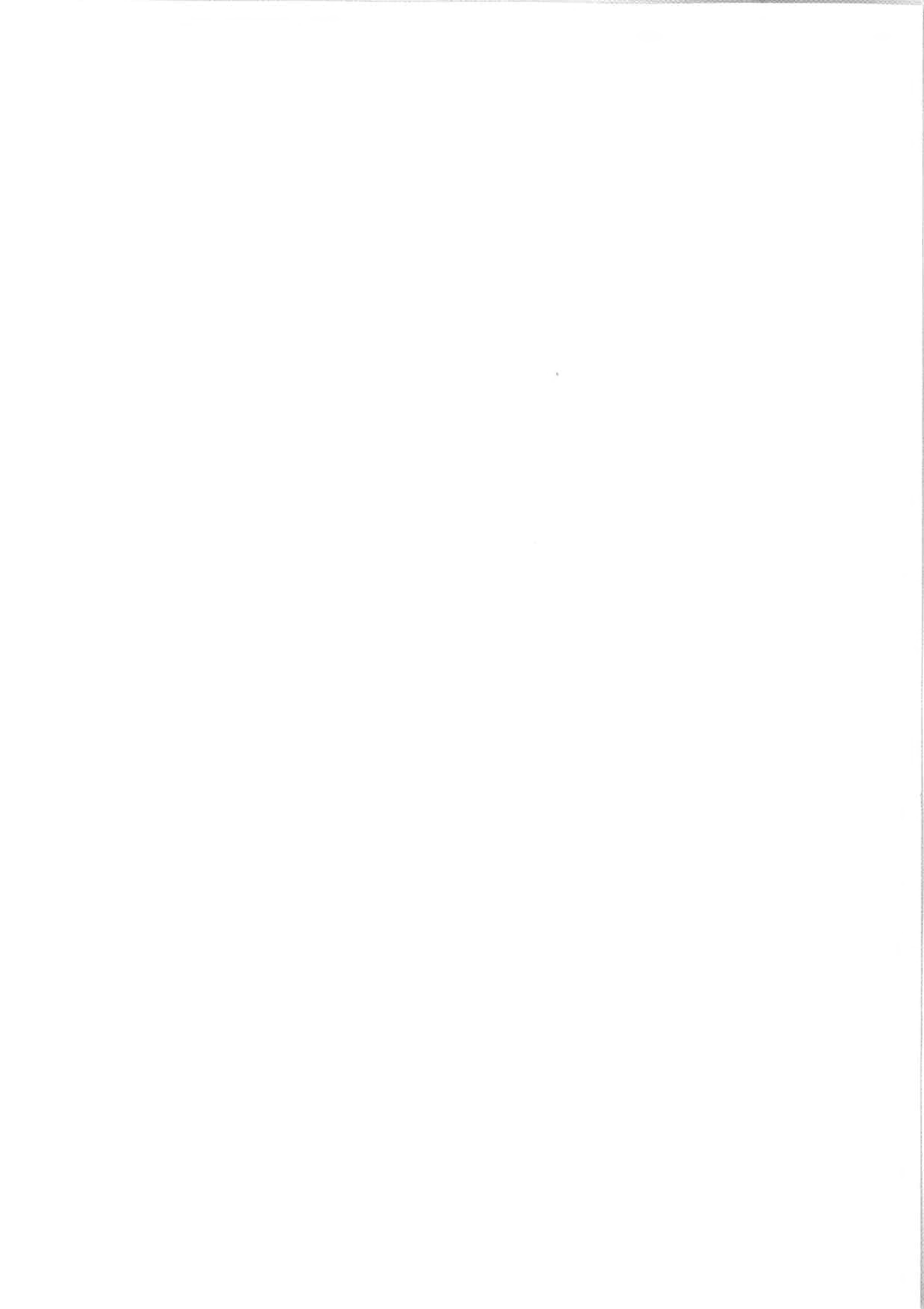
We also defined a set of n Boolean (binary) variables $^1x, ^2x, \dots, ^nx$ to indicate whether the n -valued variable x is above a specific threshold ($^kx = 1$ means $x \geq k$). These variables are used in writing the logical relations.

Multivalued *functions* were trickier to define. For the logical sum, our procedure involves assigning a specific weight to each term in the logical relation. The weighted algebraic sum is then "discretized" according to the scale of thresholds of the corresponding variable, so an n -valued variable will be associated with an n -valued function. The integers resulting from the discretization of certain weights or sums of weights are called *logical parameters*. For appropriate values of these parameters, the generalized sum gives the results of the classical logical product, so this unique generalized operation includes both classical operations.

Generalized kinetic logic — although retaining the analytic simplicity of the naïve description — has certain features in common with the differential description. These analogies are discussed in detail in Chapter 8. It is important to realize, however, that generalized logical relations are completely independent of the differential description and can be derived directly from the graph of interactions or from a sufficiently explicit verbal description. The state tables are then readily constructed in terms of the logical parameters, and for any set of values of these parameters, it is a straightforward matter to determine the behavior of the system.

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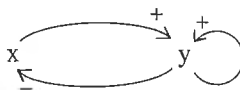


GENERALIZED LOGICAL VS. DIFFERENTIAL DESCRIPTION**TABLE OF CONTENTS**

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I. INTRODUCTION

In Chapter 7, Section V, we presented a generalized logical description of the system

described by the graph , and chose several sets of logical parameter values for further analysis. In the present chapter, we will (1) present a differential description of the same system, (2) show how the logical analysis can be used to choose real parameter values giving similar behavior in the continuous system, and (3) compare the results of the logical and differential descriptions, using sigmoids for the latter with $n \rightarrow \infty$, $n = 20$, and $n = 3$. Our presentation is not mathematically rigorous; for demonstrations, the reader is referred to Snoussi.¹

II. DIFFERENTIAL AND LOGICAL DESCRIPTIONS

For the differential description, we will use the same graph of interactions as for the generalized logical description, without specifying the nature of the connection between the two

interactions regulating element y ($x \xrightarrow{+} y$ and $y \xrightarrow{+} y$). This was justified in Chapter 7,

where we saw that the generalized logical sum includes the classical sum and the classical product, according to the values of the logical parameters, i.e., according to the weights assigned to the various terms. In the differential description, the situation is much the same: each term is assigned a characteristic weight k_{ij} (of the j^{th} element on the i^{th} element). We therefore describe our system by the differential equations:

$$\begin{aligned} \frac{dx}{dt} &= \frac{k_{12}\vartheta_{12}^n}{\vartheta_{12}^n + y^n} - k_{-1}x \\ \frac{dy}{dt} &= \frac{k_{21}x^n}{\vartheta_{21}^n + x^n} + \frac{k_{22}y^n}{\vartheta_{22}^n + y^n} - k_{-2}y \end{aligned} \quad (1)$$

in which, as in Chapter 7, the blank space in the first equation denotes that in this system x does not affect its own regulation ($k_{11} = 0$). As usual, the k_{ij} and k_{-i} are positive kinetic constants reflecting synthesis and decay, respectively.

If the sigmoids used become infinitely steep ($n \rightarrow \infty$), we get piecewise linear equations.² For $\vartheta_{12} < \vartheta_{22}$:

$$\begin{aligned} \frac{dx}{dt} &= k_{12}\overline{y} - k_{-1}x \\ \frac{dy}{dt} &= k_{21}^I x + k_{22}^2 y - k_{-2}y \end{aligned} \quad (2)$$

in which the sigmoids of Equation (1) have become step functions and are replaced by dimensionless Boolean variables.

The type of piecewise differential equation we use represents a generalization of those used by Glass in three respects:

1. Instead of using one Boolean expression B_i with one coefficient k_i as in $dx_i/dt = k_i B_i(x_1, x_2, \dots, x_n) - k_{-i} x_i$, we frequently use equations whose regulatory part is a sum of Boolean expressions, each with its own coefficient, as in the second equation of (2). This, of course, is how we assign a characteristic weight to each interaction.
2. We include self input in our description. In the example chosen, element y exerts a positive effect on its own synthesis.
3. When an element acts at more than one level, we associate a distinct threshold with each interaction. As a result, we have to use more than one Boolean variable to describe such elements in the piecewise linear differential equations. For example, here we use the Boolean variables 1y (which has the value 1 for $y > \vartheta_{12}$) and 2y (which has the value 1 for $y > \vartheta_{22}$).

Before directly comparing the differential and logical descriptions, a few words are in order on the correspondence we might expect between the differential function $H(x, y, z, \dots) = dx/dt$ and our logical function X . Consider a logical system that includes the relation:

$$X = \phi(x, y, z, \dots).$$

As explained in Chapter 3, each logical state, described by the variables x, y, z, \dots , has an image, described by the functions X, Y, Z, \dots , which is nothing more than the state which would be reached next if the operation ϕ were applied to the present state of the system, in other words, if all the orders applied to this state were executed. For example, in the system of Chapter 4, Section III, the image of state $\bar{0}\bar{0}\bar{0}\bar{0}$ is 1111. This image would be the state following $\bar{0}\bar{0}\bar{0}\bar{0}$ only if all four orders were executed synchronously, which is normally not the case.

The logical equivalent of the derivative is not X itself but the algebraic difference $X - x$; in the situation $\bar{0}/1$ (in which the product is appearing and the derivative is positive) this difference is +1; in the situation $\bar{1}/0$ (in which the product is disappearing and the derivative is negative) this difference is -1.

Consider now the differential equation:

$$\frac{dx}{dt} = k_1 F_1(x, y, z, \dots) - k_{-1} x = H(x). \quad (3)$$

We know that, in general, it cannot be integrated analytically. To identify the trajectories, we have to resort to numerical iterations, which are all variants of the Euler formula (see Appendix 2):

$$x_{n+1} = x_n + h H(x_n) \quad (4)$$

Rewriting equation (3):

$$x + \frac{1}{k_{-1}} \frac{dx}{dt} = \frac{k_1}{k_{-1}} F(x, y, z, \dots) \quad (5)$$

it can be seen that (1) the left member of Equation (5) is formally identical to the right member of Equation (4), with $h = 1/k_{-1}$; $x + (1/k_{-1}) dx/dt$ is thus the next value of x in a Euler iteration with a step $h = 1/k_{-1}$. (2) The right member of Equation (5), $(k_1/k_{-1}) F(x, y, z, \dots)$, is an algebraic description of the influences acting on x , in exactly the same way as $\phi(x, y, z, \dots)$ is the logical description of these influences.

iteration with a step $h = 1/k_{-1}$. (2) The right member of Equation (5), $(k_1/k_{-1}) F(x, y, z, \dots)$, is an algebraic description of the influences acting on x , in exactly the same way as $\phi(x, y, z, \dots)$ is the logical description of these influences.

It should now be clear, from the parallel between

$$X = \phi(x, y, z, \dots)$$

and

$$X = x + \frac{1}{k_{-1}} \frac{dx}{dt} = \frac{k_1}{k_{-1}} F(x, y, z, \dots) \quad (5)$$

that *the logical function X is the discrete counterpart of the differential expression $x + (1/k_{-1}) dx/dt$* .^{3,4}

Accordingly, Snoussi's discretization of the piecewise linear differential equations (2) (in which $\vartheta_{12} < \vartheta_{22}$) generates the following logical relations:

$$X = d_x \frac{k_{12}}{k_{-1}} \overline{1} y$$

$$Y = d_y \left(\frac{k_{21}}{k_{-2}} 1 x + \frac{k_{22}}{k_{-2}} 2 y \right)$$

Comparing these relations with those of Chapter 7, it can be seen that the weights (K s) assigned to the terms in the generalized logical description correspond to the (k/k_{-}) s. These values, it will be recalled, represented the boundaries of the differential system, i.e., they delimit the region of the variable space toward which the system will always move from outside.

In the logical description (Chapter 7, Section V), we chose several sets of values of the logical parameters and analyzed the resulting patterns of behavior. The analysis was straightforward and could be extended with little effort to all 28 possible combinations of parameter values, thus establishing the complete catalog of behavioral patterns of the system.

Similarly, in the differential description, we would like to know all possible patterns of behavior of the system and, for each one, the range of parameter values for which it will occur. For any given set of parameter values, it is relatively easy to calculate the steady-state values and to determine the stability and mode of approach of each steady state (see Chapter 6), but there is no general algorithm for extracting *all* possible behavioral patterns directly from the differential equation.

Our logical analysis is basically a formalization of intuitive reasoning, with the advantage that one can be certain of not missing any behavioral patterns, no matter how unexpected or counter-intuitive they may be. Although strictly speaking, the steady-state values of the differential equations are not in the domain of the prediction of the logical analysis, one can take advantage of the logical state table to predict without calculation the approximate location of the steady states in the corresponding continuous system. This is best illustrated by an example. The state table of the above system is

02/0, $d_y(K_{22})$	12/0, $d_y(K_{21} + K_{22})$
01/0, 0	11/0, $d_y(K_{21})$
00/ $d_x(K_{12})$, 0	10/ $d_x(K_{12})$, $d_y(K_{21})$

The logical state 02/0, $d_y(K_{22})$ will be a stable state if $d_y(K_{22}) = 2$. In this case, we can expect a *stable steady state* in the differential description. Where will it be? The value of x^0 will be near 0. As for y^0 , it will be near K_{22} , i.e., k_{22}/k_{-2} , because for values of y greater than this boundary, the derivative dy/dt is negative, whereas for $\vartheta_{22} < y < K_{22}$, it is positive. The stable state is thus expected near $(0, k_{22}/k_{-2})$. Similarly, if $d_x(K_{12}) = d_y(K_{21}) = 1$, the logical

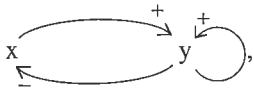
description predicts the cycle $00 \longrightarrow 10 \longrightarrow 11 \longrightarrow 10$, which reflects homeostatic

oscillation around the threshold values ϑ_{12} and ϑ_{21} . In the differential description, we expect to find a *focus* in the vicinity of $(\vartheta_{12}, \vartheta_{21})$, whenever $K_{12} > \vartheta_{21}$ and $\vartheta_{12} < K_{21} < \vartheta_{22}$.

If all three of the above equalities hold, *both* steady states are found (as in Case 2 of Chapter 7). In addition, there is a third, unstable steady state: a saddle point located near $(0, \vartheta_{22})$, on the separatrix. Identification of this type of unstable steady state on logical grounds requires a slightly more elaborate analysis (see Section V).

The correspondence between logical and differential steady states and between logical cycles and differential foci has been established by Snoussi¹ for piecewise linear differential equations (in which the Hill functions become step functions) and their discretized counterparts. As we shall see, the correspondence remains qualitatively satisfactory down to rather low values of n in the Hill functions.

III. COMPARISON OF LOGICAL AND DIFFERENTIAL DESCRIPTIONS

We will now return to our concrete two-element example, , and compare the generalized logical description, using four of the sets of logical parameter values treated in Chapter 7, with the differential description, using differential parameter values predicted to give the same pattern of behavior. We will first recall the four logical tables and use them to choose parameter values for the differential description (A); we will then analyze the four resulting differential descriptions (B), and finally, look at the trajectories followed by the differential system (C).

A. USE OF THE LOGICAL TABLES TO PREDICT DIFFERENTIAL PARAMETERS

Although in the generalized logical treatment multivalued functions and variables are used, they are still discrete, taking only the values 0, 1, 2, etc. Each variable has its own scale, corresponding to the intervals between threshold values for the various interactions of the corresponding element. For example, $x = 1$ represents a value greater than the first threshold on the x scale and less than the second. Similarly, the values assigned to the logical parameters represent inequalities, placing each parameter in a specific interval on the appropriate scale.

The differential description, on the other hand, uses real variables and continuous functions, and for numerical work each parameter must be assigned a precise value. The inequalities dictated by the logical image serve as a guide, but can, of course, be satisfied in an infinite number of ways, so there remains a degree of arbitrariness in the choice of differential parameters.

In what follows, we will set $k_{-1} = 2$, $k_{-2} = 1$ and $\vartheta_{12} = 1$ in all cases. For the remaining parameters, we will choose values compatible with the inequalities dictated by the logical

TABLE 1

	Case 1	Case 2	Case 3	Case 11
K_{12}	1	1	1	1
K_{21}	0	1	2	2
K_{22}	2	2	2	1
k_{-1}	$\begin{bmatrix} 2 \\ 1 \end{bmatrix}$	$\begin{bmatrix} 2 \\ 1 \end{bmatrix}$	$\begin{bmatrix} 2 \\ 1 \end{bmatrix}$	$\begin{bmatrix} 2 \\ 1 \end{bmatrix}$
ϑ_{ij}	$\begin{bmatrix} - & 1 \\ 3 & 4 \end{bmatrix}$	$\begin{bmatrix} - & 1 \\ 3 & 4 \end{bmatrix}$	$\begin{bmatrix} - & 1 \\ 3 & 4 \end{bmatrix}$	$\begin{bmatrix} - & 1 \\ 2 & 1 \end{bmatrix}$
k_{ij}	$\begin{bmatrix} - & 12 \\ 0.5 & 8 \end{bmatrix}$	$\begin{bmatrix} - & 12 \\ 2 & 8 \end{bmatrix}$	$\begin{bmatrix} - & 12 \\ 7 & 8 \end{bmatrix}$	$\begin{bmatrix} - & 8 \\ 2 & 0.9 \end{bmatrix}$
$K_{ij} = k_{ij}/k_{-1}$	$\begin{bmatrix} - & 6 \\ 0.5 & 8 \end{bmatrix}$	$\begin{bmatrix} - & 6 \\ 2 & 8 \end{bmatrix}$	$\begin{bmatrix} - & 6 \\ 7 & 8 \end{bmatrix}$	$\begin{bmatrix} - & 4 \\ 2 & 0.9 \end{bmatrix}$

parameters of the system whose behavior we are trying to mimic. In Cases 1, 2, and 3, the logical parameters K_{12} and K_{22} have their maximal value (1 and 2, respectively). This

means that the terms concerning the interactions $y \xrightarrow{-} x$ and $y \xrightarrow{+} x$ are both strong (cf. Chapter 7). Translated into the differential system, for these three cases we must choose parameter values that respect the inequalities $K_{12} > \vartheta_{21}$ (i.e., $k_{12}/k_{-1} > \vartheta_{21}$) and $K_{22} > \vartheta_{22}$ (i.e., $k_{22}/k_{-2} > \vartheta_{22}$). We will set $K_{12} = 12$, $\vartheta_{21} = 3$, and $K_{22} = 8$, $\vartheta_{12} = 1$, $\vartheta_{22} = 4$ for these cases. The difference lies in the value of the logical parameter K_{21} , which is 0, 1, and 2 for Cases 1, 2, and 3, respectively. This corresponds to increasing the weight of the term concerning the interaction $x \xrightarrow{+} y$, and, consequently, to increasing the strength of the

negative loop $x \xrightarrow{+} y \xrightarrow{-} x$.

Case 1 — With $K_{21} = 0$ in the logical description, we should have $K_{21} < \vartheta_{12}$ (i.e., $k_{21}/k_{-2} < \vartheta_{12}$); we will set $K_{21} = 0.5$. The logical table presents two stable states, $\textcircled{02}$ and $\textcircled{10}$. Their expected location in the differential description is $(0, k_{22}/k_{-2})$ and $(k_{12}/k_{-1}, k_{21}/k_{-2})$, i.e., $(0, 8)$ and $(6, 0.5)$, respectively, with a separatrix between the domains located close to $y = \vartheta_{22}$, i.e., $y = 4$. The actual locations of the steady states are given in Table 2.

Case 2 — With $K_{21} = 1$, we want $\vartheta_{12} < K_{21} < \vartheta_{22}$; we will set the real parameter $K_{21} = 2$. The logical table presents a stable state, $\textcircled{02}$, and a cycle, $00 \rightarrow 10 \rightarrow 11 \rightarrow 01$. From this, we can expect the differential image to have a stable state near $(0, k_{22}/k_{-2})$, i.e., $(0, 8)$, and a focus near $(\vartheta_{21}, \vartheta_{12})$, i.e., $(3, 1)$, with a separatrix between the domains, located close to $y = \vartheta_{22}$, i.e., $y = 4$ (see Table 2).

TABLE 2

Predicted	Calculated	Roots (ω) of characteristic equation	Nature of the steady state
1. $(0, K_{22})$ $n \rightarrow \infty$	$(0, 8)$		
$n = 20$	$(5 \times 10^{-18}, 7.99)$	$-0.999, -2$	Stable node
$n = 3$	$(0.022, 6.47)$	$-0.4272, -1.999$	Stable node
$(0, \vartheta_{22})^a$ $n \rightarrow \infty$	$(0, 4)$		
$n = 20$	$(5.4 \times 10^{-12}, 4)$	$9, -2$	Saddle point
$n = 3$	$(0.092, 3.99)$	$0.499, -1.99$	Saddle point
(K_{12}, K_{21}) $n \rightarrow \infty$	$(6, 0.5)$		
$n = 20$	$(5.99, 0.499)$	$-1, -1.99$	Stable node
$n = 3$	$(5.52, 0.441)$	$-1.15, -1.77$	Stable node
2. $(0, K_{22})$ $n \rightarrow \infty$	$(0, 8)$		
$n = 20$	$(5 \times 10^{-18}, 7.99)$	$-0.999, -2$	Stable node
$n = 3$	$(0.022, 6.47)$	$-0.427, -1.999$	Stable node
$(0, \vartheta_{22})^a$ $n \rightarrow \infty$	$(0, 4)$		
$n = 20$	$(5.4 \times 10^{-12}, 4)$	$9, -2$	Saddle point
$n = 3$	$(0.092, 3.99)$	$0.499, -1.99$	Saddle point
$(\vartheta_{21}, \vartheta_{12})$ $n \rightarrow \infty$	$(3, 1)$		
$n = 20$	$(3.00, 1.00)$	$-1.49 \pm 14i$	Stable focus
$n = 3$	$(2.81, 1.04)$	$-1.30 \pm 2i$	Stable focus
3. $(0, K_{22})$ $n \rightarrow \infty$	$(0, 8)$		
$n = 20$	$(5 \times 10^{-18}, 7.99)$	$-0.999, -2$	Stable node
$n = 3$	$(0.022, 6.47)$	$-0.427, -1.999$	Stable node
$(0, \vartheta_{22})^a$ $n \rightarrow \infty$	$(0, 4)$		
$n = 20$	$(5.4 \times 10^{-12}, 4)$	$9, -2$	Saddle point
$n = 3$	$(0.092, 3.99)$	$0.499, -1.99$	Saddle point
$(\vartheta_{21}, \vartheta_{12})$ $n \rightarrow \infty$	$(3, 1)$		
$n = 20$	$(2.74, 1.008)$	$-1.49 \pm 19.2i$	Stable focus
$n = 3$	$(1.68, 1.36)$	$-1.17 \pm 2.79i$	Stable focus
11. $(\vartheta_{21}, \vartheta_{12})$ $n \rightarrow \infty$	$(2, 1)$		
$n = 20$	$(1.901, 1.005)$	$0.733 \pm 12.4i$	Unstable focus
$n = 3$	$(1.528, 1.173)$	$-1.288 \pm 1.85i$	Stable focus

^a Predicted by the method described in Section V.

Case 3 — Here, $K_{21} = 2$, so $K_{21} > \vartheta_{22}$; we will set $K_{21} = 7$. The logical table is similar to that of Case 2, except that the variable space is not separated into two distinct parts, and from state $\bar{1}\bar{1}$ the system can proceed either to $0\bar{1}$ (and follow the cycle) or to $\textcircled{02}$ (via $\bar{1}2$) (see Table 2). The locations predicted for the steady states are as in Case 2.

Case 11 — Here, the two thresholds of element y coincide ($\vartheta_{12} = \vartheta_{22}$). The values of the logical parameters are $K_{12} = 1$, $K_{21} = 1$, and $K_{22} = 0$ (cf. Chapter 7). In other words, the

terms involving the negative loop $x \begin{array}{c} \xrightarrow{+} y \\ \xleftarrow{-} x \end{array}$ are strong, but the weight given to the

term concerning the autocatalytic interaction $y \xrightarrow{+}$ is too little to impose multistationarity by itself. This is what we represent in Chapter 7 by the graph $x \xrightarrow{+} y \xrightarrow{+} x$. In the differential description, we must have $K_{22} < \vartheta_{22}$. We will set $\vartheta_{12} = \vartheta_{22} = 1$, $\vartheta_{21} = 2$, and $K_{22} = 0.9$. The logical image is a cycle, which suggests that the differential description will have a focus located near $(\vartheta_{21}, \vartheta_{12})$ (see Table 2).

B. DIFFERENTIAL DESCRIPTION

We will now examine the behavior of continuous systems designed to mimic the four discrete systems by using the above parameter values (see Table 1). In Figure 1 are shown the nullclines of the four sets of differential equations, using $n = 20$. The steady states are seen at the intersections of the nullclines $dx/dt = 0$ and $dy/dt = 0$. The nature of the steady states can also be determined by linear stability analysis, as described in Chapter 6 and Appendix 3. For $n \rightarrow \infty$, steady states are found precisely at the locations predicted by the logical analysis (Table 2). They are quite close for $n = 20$ and even for $n = 3$.

As for the *nature* of these steady states, those corresponding to the cycles in the logical description are, as expected, foci with a strong periodic component (the roots of the characteristic equation have a high imaginary part). This is particularly clear in Case 11, whose unique steady state is a focus. The steady states corresponding to logical stable states are stable nodes, with negative real roots.

It can be seen in Figure 1 that for Cases 1, 2, and 3, there is also a third steady state that we have not mentioned. Linear stability analysis shows it to be a saddle point. In fact, its location can also be predicted on logical grounds, but, as mentioned above, this requires a slightly more elaborate analysis (see Section V).

C. TRAJECTORIES

In Figure 2 are shown the trajectories for the continuous systems described above. In Cases 1, 2, and 3, two initial states have been chosen such that, although they are extremely close, their trajectories ultimately diverge, one leading to the upper steady state and the other to the lower one. In Case 1, the pathway leads without periodicity to the upper or lower steady state, essentially according to whether the initial value of y is "high" or "low". In Case 2, again the initial value of y determines which steady state is approached, but here the lower one is approached periodically. In Case 3, the choice is between a stable node and a stable focus, but the upper steady state can be approached even from initial points with y low or zero, provided x is high enough. All this fits remarkably well with the logical description; even for n as low as 3, the essential qualitative aspects of the logical description are preserved.

Case 11 is of special interest for the following reason. As pointed out in Chapter 6, our logical analysis detects foci (as in this case), but does not say whether they are stable or unstable. As a matter of fact, Figure 2 and Table 1 show that for the parameter values chosen, the unique steady state in Case 11 is indeed a focus, which is stable for $n = 3$, but unstable for $n = 20$, resulting in a limit cycle. This is the first time we have encountered a limit cycle in a two-variable system. It will be remembered that for a simple two-element feedback loop, we have demonstrated that the focus is necessarily stable (in the absence of time delays). How does it happen, then, that in Case 11, we find an unstable focus with a limit cycle in a two-variable system? This point will be examined in Chapter 16, where we discuss the respective roles of negative loops in periodicity and of positive loops in multistationarity and stabilization of the periodicity.

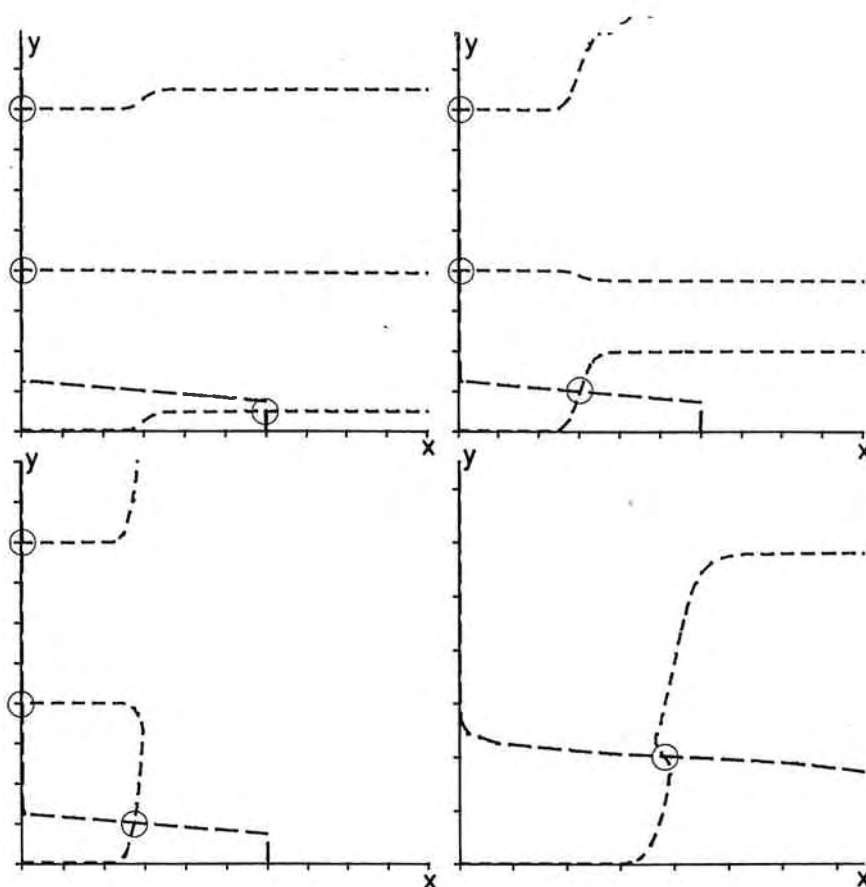


FIGURE 1. The nullclines of Cases 1, 2, and 3 for $n = 20$. As usual, long dashes are used for the nullclines $dx/dt = 0$ and short dashes for $dy/dt = 0$.

IV. BASAL LEVELS AND REGULATED DESTRUCTION

A. BASAL LEVELS OF GENE EXPRESSION

So far, we have reasoned as if a gene under positive control were not expressed at all in the absence of its positive regulator, and as if a gene under negative control were not expressed at all in the presence of a sufficient amount of repressor. However, this is usually not the case; the residual expression is then called the *basal level* of expression of the gene.

The differential description can readily include the basal level. For a gene X positively controlled by product y , for example, to the rate equation

$$\frac{dx}{dt} = k_1 F^+(y) - k_{-1}x,$$

in which the synthesis term is zero when $y = 0$, we add a constant term k_0 (independent of y) to represent the basal level:

$$\frac{dx}{dt} = k_0 + k_1 F^+(y) - k_{-1}x.$$

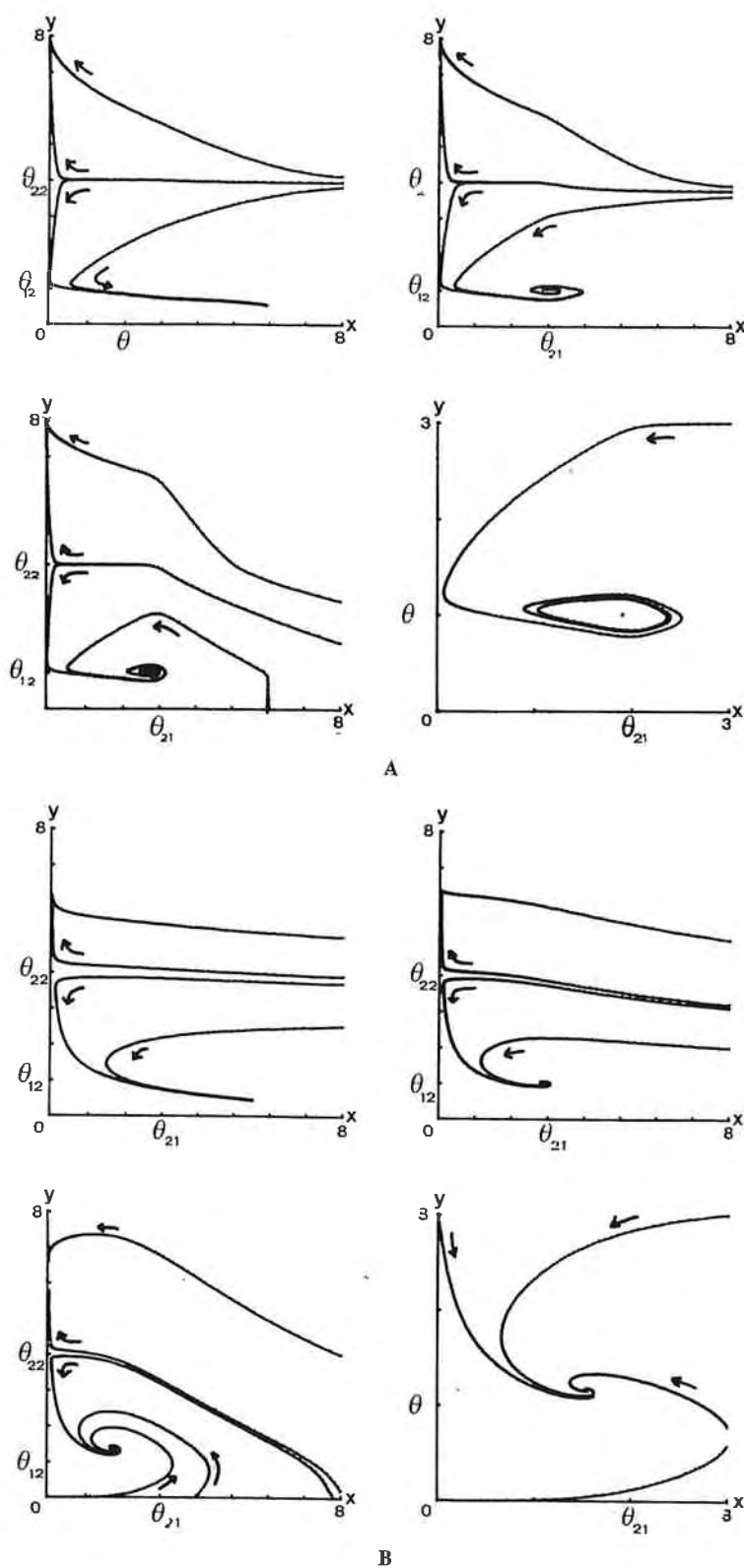


FIGURE 2. Trajectories for Cases 1, 2, 3, and 11 for $n = 20$ and $n = 3$. For Cases 1, 2, and 3, two of the trajectories shown have extremely close initial states, but nevertheless eventually diverge.

The generalized logical description will thus be

$$X = d_x (K_0 + K_1 y)$$

with $K_0 = k_0/k_{-1}$ and $K_1 = k_1/k_{-1}$. Taking into account the fact that gene \underline{X} might be mutationally inactivated, we have

$$X = g_x d_x (K_0 + K_1 y)$$

where g_x can take the value 1 (gene normal) or 0 (gene mutationally inactivated). The state table (in which we take g_x as an input variable) is

X	0	1	g_x
0	0	$d_x(K_0)$	
1	0	$d_x(K_0 + K_1)$	
y			

If $d_x(k_0) = 0$, then the basal level of \underline{x} has no physiological significance and is formally equivalent to a complete absence of product \underline{x} . Similarly, if $d_x(K_0) = d_x(K_0 + K_1)$, the basal level is formally equivalent to the fully stimulated level (at high \underline{y} concentration). Obviously, if element \underline{x} interacts with only one other element and thus has only a single threshold (making \underline{x} a binary variable), one of these conditions will hold. If, on the other hand, \underline{x} has two thresholds, ϑ_1 and ϑ_2 , and

$$\vartheta_1 < d_x(K_0) < \vartheta_2 < d_x(K_0 + K_1)$$

the state table becomes:

X	0	1	g_x
0	0	1	
1	0	2	
y			

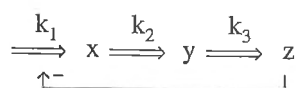
0	ϑ_1	ϑ_2	X
	$d_x(K_0)$	$d_x(K_0 + K_1)$	
	$(K_0 = 1)$	$(K_0 + 1 = 2)$	

In this case the basal level of expression ($x = 1$) is different from both the fully stimulated level ($x = 2$) and the complete absence of product ($x = 0$). An example of this pattern of expression is the RecA protein of *Escherichia coli*, whose basal level is required for induction of the SOS response and whose stimulated level is required to induce a λ prophage (cf. Chapter 19).

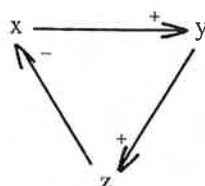
B. REGULATED DESTRUCTION OF A PRODUCT

So far we have reasoned as though the decay of an element were strictly proportional to the concentration of the element, making it a linear term in the differential description. This term accounts for the unregulated, aspecific decay of the element into unspecific byproducts.

It may also include a linear conversion of the element into another element of the system. For example, in the system:



product x is converted into y and y into z , which is a negative regulator of the synthesis of x . (The double arrows indicate chemical conversion and the single arrow indicates a regulatory interaction.) The graph of interactions is



and the differential equation for the synthesis of x is

$$\begin{aligned} \frac{dx}{dt} &= k_1 F^-(z) - k_2 x - k_{-1} x \\ &= k_1 F^-(z) - (k_2 + k_{-1})x \end{aligned} \quad (6)$$

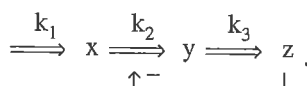
The conversion of x into y is represented by the linear term $-k_2 x$, which can be combined with the spontaneous decay term, $-k_{-1} x$.

The generalized logical description is thus:

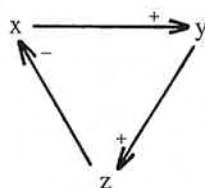
$$X = d_x (K_1 \bar{z}),$$

with $K_1 = k_1/(k_2 + k_{-1})$.

Let us now consider a similar system in which the negative effect exerted by product z on x is not via inhibition of the synthesis of x , but by destruction of x , converting it to y :



This system has the same graph of interactions as the preceding one:



although the molecular *mechanism* of the negative interaction is entirely different. If the "anti- x " activity of z has a sigmoid dependence on z concentration (i.e., essentially no activ-

ity up to a critical threshold and an upper limit at high \underline{z} concentration), the differential equation for \underline{x} synthesis is

$$\frac{dx}{dt} = k_1 - k_2 F^+(z) - k_{-1}x \quad (7)$$

This raises two questions. First, when the graphs of interactions (and our intuition) tell us that the two systems are formally equivalent, how can we justify the replacement of the term $+k_1 F^-(z)$ in Equation (6) by the terms $k_1 - k_2 F^+(z)$ in Equation (7)? And second, how do we translate Equation (7) into our generalized logical formalism? The answers to both questions become obvious if we recall the identity from Chapter 6:

$$F^-(z) = 1 - F^+(z),$$

or

$$\frac{\vartheta^n}{x^n + \vartheta^n} = 1 - \frac{x^n}{x^n + \vartheta^n}.$$

Equation (7) can thus be written:

$$\frac{dx}{dt} = (k_1 - k_2) + k_2 F^-(z) - k_{-1}x,$$

which has the same form as Equation (6), apart from a constant term (which reflects the fact that the second system has a basal level of synthesis of \underline{x} even in the presence of high concentrations of \underline{z}). The corresponding logical relation is now seen to be

$$X = d_x (K_1 - K_2 + K_2 \bar{z}).$$

The two systems are thus seen to have the same logical structure, in agreement with the intuitive feeling that they are merely different mechanisms to achieve the same goal.

A concrete example of a system in which the active degradation of one of the elements is part of the regulatory circuit is encountered in the SOS response in *E. coli*, in which the RecA protein specifically degrades certain repressors when DNA replication is perturbed. This system is discussed in Chapter 19.

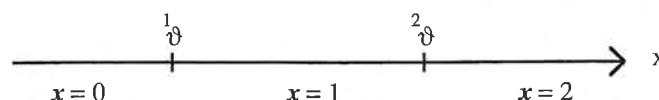
V. LOGICAL IDENTIFICATION OF THE LOCATION OF ALL STEADY STATES

When we find a logical state for which the variable vector and the function vector have the same values, we call it a (logical) stable state. We conjectured that each logical stable state is represented by a stable steady state in homologous differential systems with sufficiently steep sigmoids. Snoussi¹ has demonstrated that this is indeed the case for $n \rightarrow \infty$ and that in this situation one finds stable nodes whose coordinates correspond to *boundary values* (0 or K_s).

So far, the other steady states of the differential description had not been identified on logical grounds; in the best cases, they could just be *inferred*. More specifically, when we have a stable cycle in the logical description, we find a focus in the differential description. However, in this case, periodicity is seen at the logical level as a cyclic sequence of logical states; no logical *state* corresponds to the focus itself. In fact, the logical equivalent of the focus is located at the junction of logical states, i.e., at *threshold* values (ϑ s). For other dif-

ferential steady states (e.g., saddle points or unstable nodes), the logical identification was even less clear, and in any case the logical equivalent, if any, was located *between* logical states.

This suggests that the logical description should not ignore the threshold values, but, rather, consider them explicitly as logical values. Consider a three-level variable:



So far we had considered only the situations:

$$x < {}^1\theta, \text{ described by } x = 0,$$

$${}^1\theta < x < {}^2\theta, \text{ described by } x = 1,$$

$${}^2\theta < x, \text{ described by } x = 2,$$

and we ignored the situations $x = {}^1\theta$ and $x = {}^2\theta$. To cover these situations, we now introduce additional logical values symbolized by ${}^1\theta$ and ${}^2\theta$, respectively. The scale is thus 0, ${}^1\theta$, 1, ${}^2\theta$, 2. It should be clear that a logical threshold level, say ${}^1\theta$, corresponds to different real values according to the variable considered.

From now on, we will call "integer" logical states the classical logical states whose representative vector includes only integers, and "noninteger" logical states, the states located on one or more thresholds.

A state located on *one* threshold is at the junction between two adjacent integer states. More generally, a state located on *n* thresholds is at the junction between 2^n adjacent integer states; for example, state ${}^1\theta {}^2\theta$ is between the integer states 01, 02, 11, and 12.

Consider now the relation: $Y = d_y(K {}^2x)$; for any $x \leq 1$ (including ${}^1\theta$ for reasons of continuity), $Y = 0$, for $x = 2$, $Y = K$, but for $x = {}^2\theta$ one cannot ascribe a defined value to Y ; all one can say here is that its value is included in $[0, K]$, and we write:

$$Y \in [0, K]$$

The extended state table (which includes the noninteger states) is thus:

x	Y
0	0
${}^1\theta$	0
1	0
${}^2\theta$	$[0, K]$
2	K

This illustrates the following point of general interest. If we consider a variable (say x) of a noninteger state, the X component of the image of this state can be well defined (as is the

case when the image of this variable is the same for all the surrounding integer states). Alternatively, the image of the variable can be an interval (or a set of intervals).

The logical steady-state equations are: $x = X$, $y = Y$, ..., and the logical states can thus be identified as the values of the vector xy ..., which are *consistent with* all of these equations. (Clearly, when the image of a variable is an *interval*, one cannot ask whether x and X are equal, but whether their values are consistent with $x = X$, that is, whether the value of x is included in the interval).

1. Let us first consider the simple, one-element positive loop: $X = d_x (K_0 + K_1 \cdot x)$. The extended state table is

x	X
0	K_0
${}^1\theta$	$[K_0, K_0 + 1]$
1	$K_0 + 1$

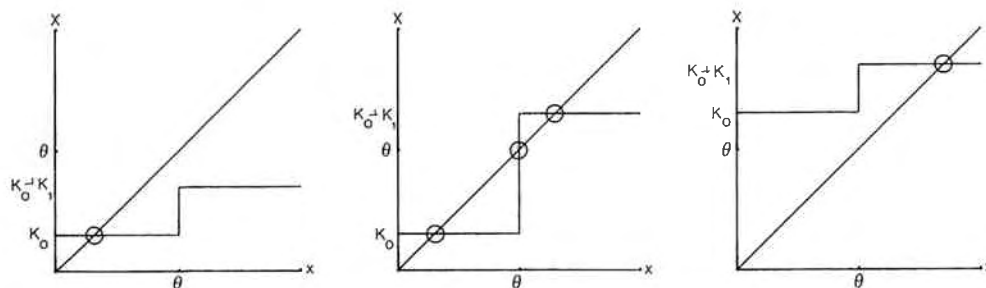
According to the values of the logical parameters, we have

x	X
①	0
1	0

x	X
①	0
①	1

x	X
0	1
①	1

with two logical stable states where $K_0 = 0$ and $K_0 + 1 = 1$. If we momentarily leave the logical description, these three situations can be visualized as follows in real space:



We see that there is multistationarity (and there is a steady state at $x = \theta$) if, and only if, $K_0 < \theta < K_0 + K_1$ (which corresponds to the logical parameter values $K_0 = 0$ and $K_0 + 1 = 1$). In other words, there is multistationarity (and there is a steady state at $x = \theta$) iff the jump from K_0 to $K_0 + K_1$ encompasses the threshold value θ .

If we now return to the logical description, we have:

$$\text{for } x = 0, \quad X = K_0$$

$$\text{for } x = 1, \quad X = K_0 + 1$$

$$\text{for } x = {}^1\theta, \quad X \in [K_0, K_0 + 1]$$

The steady-state equation is $x = X$. Thus,

$x = 0$ is a steady state iff $K_0 = 0$,

$x = 1$ is a steady state iff $K_{0+1} = 1$, and

$x = {}^1\theta$ is a steady state iff ${}^1\theta \in [K_0, K_{0+1}]$.

that is, iff the jump from K_0 to K_{0+1} encompasses the threshold value ${}^1\theta$.

Clearly, if $K_0 = 0$ and $K_{0+1} = 1$, $x = 0$ and $x = 1$ are both (stable) steady states, and $x = {}^1\theta$ is also a steady state because ${}^1\theta \in [0, 1]$. Otherwise, we have only one steady state, either $\textcircled{0}$ or $\textcircled{1}$. The fit with differential description is perfect; the steady state at $x = {}^1\theta$ is the logical equivalent of the unstable steady state found at $x = \theta$ in the differential description.

2. Consider now a slightly more elaborate system — a two-variable system with a single, one-element loop:

$$\begin{cases} X = d_x(K_0 + K_{11} {}^2x) \\ Y = d_y(K_{21} {}^1x) \end{cases} \quad \text{or,} \quad \begin{array}{c} \textcircled{+2} \text{ } x \xrightarrow{+} y \\ \quad \quad \quad \uparrow + \\ \quad \quad \quad y \end{array}$$

which can be symbolized more simply by the matrix:

$$\begin{pmatrix} \textcircled{+2} & 0 \\ +1 & 0 \end{pmatrix}$$

A look at the matrix shows that there is a positive feedback loop of x , acting on itself at threshold ${}^2\theta$ (in short, a loop “ 2x ”, circled in the matrix) and that y exerts no feedback whatsoever; moreover, it is seen that x exerts its effect on Y if $x \geq 1$ and on X only if $x = 2$. The state table is

x	X	Y
0	K_0	0
${}^1\theta$	K_0	$[0, K_{21}]$
1	K_0	K_{21}
${}^2\theta$	$[K_0, K_{0+1}]$	K_{21}
2	K_{0+11}	K_{21}

For any $x \leq 1$, $X = K_0$

For $x = 2$, $X = K_{0+11}$

For $x = {}^2\theta$, $X \in [K_0, K_{0+11}]$

If $k_0 \leq 1$ and $K_{0+11} = 2$, $X \in [0, 2]$ and hence, ${}^2\theta \in [0, K_{0+11}]$ in agreement with the steady state equation $x = X$. According to whether $K_{21} = 0$ or 1, the corresponding steady state will be $({}^2\theta 0)$ or $({}^2\theta 1)$ (and the third steady state (20) or (21)).

For steady states located on two or more thresholds, a rigorous treatment requires additional considerations, which will not be developed here (but see Thomas⁵). We will nevertheless briefly consider some simple cases:

3. A simple, two-element positive loop.

$$\begin{cases} X = d_x(K_{12} \bar{y}) \\ Y = d_y(K_{21} \bar{x}) \end{cases} \quad \text{or,} \quad \begin{array}{c} \xrightarrow{\quad} y \\ x \xleftarrow{\quad} \end{array}$$

which can be symbolized more simply by the matrix:

$$\begin{pmatrix} 0 & -1 \\ -1 & 0 \end{pmatrix}$$

The positive loop between x and y , acting at their threshold ${}^1\theta$ (in short, the loop " $\bar{x} \bar{y}$ ") is circled. The state table is

x	y	X	Y
0	0	K_{12}	K_{21}
0	1	0	K_{21}
1	0	K_{12}	0
1	1	0	0

(with two stable states (01) and (10) if K_{12} and K_{21} both equal 1, and only one stable state if either or both of the K s equal 0).

We see that:

$$\begin{array}{ll} \text{for } x = 0 & Y = K_{21} \\ \text{for } x = 1 & Y = 0 \\ \text{for } x = {}^1\theta & Y \in [0, K_{21}] \end{array}$$

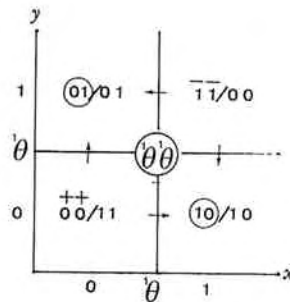
Similarly,

$$\begin{aligned}
 \text{for } y = 0 & \quad X = K_{12} \\
 \text{for } y = 1 & \quad X = 0 \\
 \text{for } y = {}^1\theta & \quad Y \in [0, K_{12}]
 \end{aligned}$$

Thus, when K_{12} and K_{21} both equal 1, state ${}^1\theta^1\theta$ is steady because $x = {}^1\theta$, $X \in [0, 1]$ is consistent with the steady equation $x = X$, and $y = {}^1\theta$, $Y \in [0, 1]$ is consistent with the steady state equation $y = Y$.

Note that the image of a state located on a threshold is generally not defined; however, if the state is a *steady* state, it has a well-defined image, identical to itself.

It is seen that for the parameter values that give multiple steady states, in addition to the steady states $\backslash O(01, \bigcirc)$ and $\backslash O(10, \bigcirc)$ already detected in the naïve description, we have a steady state $\bigcirc({}^1\theta^1\theta)$, which, in fact, corresponds to the saddle point of the differential description:



Could there be other steady states located on thresholds in this system? For example, could $0^1\theta$ be a steady state? This state is located between the integer states 00 and 01. The “local” state table (which gives the integer states adjacent to the noninteger state considered) is

$x y$	X	Y
00	K_{12}	K_{21}
$0^1\theta$	$[0, K_{12}]$	K_{21}
01	0	K_{21}

One sees that (for reasons of continuity) for $y = {}^1\theta$, $Y = K_{21}$. Thus, whatever the (integer) value of K_{21} , one cannot have $y = Y$, and state $0^1\theta$ cannot be steady.

A screening of all the noninteger states of the system would show that ${}^1\theta^1\theta$ is indeed the only possible noninteger steady state of this system, and that it is steady if and only if K_{12} and $K_{21} = 1$ (and not K_{12} and $K_{21} \geq 1$, because in this system 1 is the maximal logical value of x, X, y, Y , and hence K_{12} and K_{21}).

Such a screening can be made “by hand” or, for more complex systems, by an existing computer program. As a matter of fact, this screening is fortunately not really necessary. As will be briefly discussed below, each feedback loop can generate (within the subspace of the

variables involved in the loop) a characteristic steady state, located at the thresholds of the loop and whose presence indicates that the loop is effective. In the present case, our positive loop " $x^I y$ " can generate a steady state at ${}^1\theta^1\theta$, and the conditions for its occurrence ($K_{12} = K_{21} = 1$) are also the conditions for the loop to be efficient, i.e., the conditions for multistationarity.

4. A simple, two-element negative loop

$$\begin{cases} X = d_x(K_{12} \bar{y}) \\ Y = d_y(K_{21} x) \end{cases} \quad \text{or,} \quad \begin{array}{c} \xrightarrow{+} \\ x \quad y \\ \xleftarrow{-} \end{array}$$

which can also be symbolized by the matrix

$$\begin{pmatrix} 0 & -1 \\ +1 & 0 \end{pmatrix}$$

in which the negative loop " $x^I y$ " is circled. The state table is

$x y$	X	Y
00	K_{12}	0
01	0	0
10	K_{12}	K_{21}
11	0	K_{21}

which has a cycle but no classical logical steady state if $K_{12} = K_{21} = 1$:

$$\begin{array}{ll} \text{for } x = 0 & Y = 0 \\ \text{for } x = 1 & Y = K_{21} \\ \text{for } x = {}^1\theta & Y \in [0, K_{21}] \end{array}$$

Similarly,

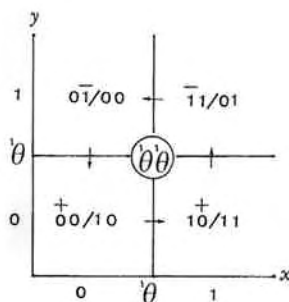
$$\begin{array}{ll} \text{for } y = 0 & X = K_{12} \\ \text{for } y = 1 & X = 0 \\ \text{for } y = {}^1\theta & X \in [0, K_{12}] \end{array}$$

Thus, when K_{12} and K_{21} both equal 1, ${}^1\theta^1\theta$ is a steady state, because for these parameter values ${}^1\theta \in [0, K_{12}]$ and ${}^1\theta \in [0, K_{21}]$, in agreement with the steady-state equations.

It is readily seen that for these values of the logical parameters, the single steady state is ${}^1\theta^1\theta$. So far, the logical description has not shown the steady state itself. Now, in addition to the logical cycle

$$00 \longrightarrow 10 \longrightarrow 11 \longrightarrow 01,$$

we see the logical steady state ${}^1\theta^1\theta$, which is the equivalent of the focus of the differential description.

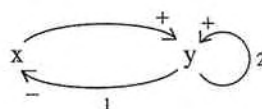


Again, a simple look at the interaction matrix can provide much information. There is no positive loop and, consequently, there can be only one steady state. For "appropriate" parameter values ($K_{12} = K_{21} = 1$), the steady state will be at ${}^1\theta^1\theta$ and the loop will be efficient (homeostasis will be ensured); otherwise, we will have a classical (integer) logical stable state.

5. We will now take the example treated in logical terms in Chapter 7 and in differential terms in the first sections of this chapter:

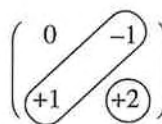
$$\begin{cases} X = d_x(K_{12}\bar{y}) \\ Y = d_y(K_{21}^1x + K_{22}^2y) \end{cases}$$

or



The interaction matrix and state table are

$x y$	X	Y
00	K_{12}	0
01	0	0
02	0	K_{22}
10	K_{12}	K_{21}
11	0	K_{21}
12	0	$K_{21} + K_{22}$



Again, we circled in the matrix the interactions that create feedback loops. There is a negative loop between variables x and y , acting both at their threshold $^1\theta$ (in short, a negative loop " $^1x^1y$ ") and a positive loop of y on itself, acting at threshold $^2\theta$ (in short, a positive loop " 2y "). We may expect their characteristic steady states at $^1\theta^1\theta$ and $^{-2}\theta$ (that is, $y = ^2\theta$ and the value of x remains to be determined), respectively. We can predict which parameter values will ensure homeostasis, which will ensure multistationarity, and which both, just by looking at which parameter values ensure the occurrence of either or both characteristic steady states.

State $^1\theta^1\theta$ is at the junction between the integer states 00, 01, 10, and 11. The local state table is

xy	X	Y
00	K_{12}	0
01	0	0
10	K_{12}	K_{21}
11	0	K_{21}

Reasoning as above, we find that the conditions for the occurrence of a steady state at $^1\theta^1\theta$ are $K_{12} = 1$ and $K_{21} = 1$. Can there be steady states of the type $^{-2}\theta$ (which would ensure multistationarity)? Let us try the three possibilities $0^2\theta$, $^1\theta^2\theta$, and $^1^2\theta$.

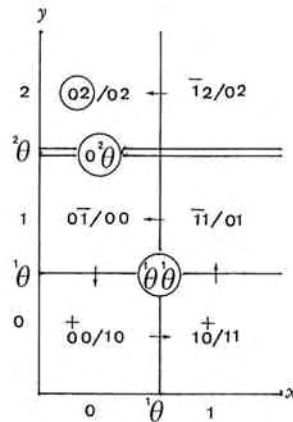
$0^2\theta$			$^1\theta^2\theta$			$^1^2\theta$		
xy	X	Y	xy	X	Y	xy	X	Y
01		00	01		00	11		$0K_{21}$
02		$0K_{22}$	02		$0K_{22}$	12		$0K_{21+22}$
			11		$0K_{21}$			
			12		$0K_{21+22}$			

Since $X = 0$ for both states 11 and 12, it is also 0 (for reasons of continuity) for state $^1^2\theta$; thus, for this state $x = 1$ but $X = 0$, and it cannot be steady. The same kind of reasoning applies for state $^1\theta^2\theta$. In contrast, state $0^2\theta$ can be steady; the only condition is that $^2\theta \in [0, K_{22}]$, that is, $K_{22} = 2$.

Thus, we see that in this system the condition for homeostasis is simply $K_{12} = K_{21} = 1$ and the condition for multistationarity, $K_{22} = 2$. The conditions to have both homeostasis and multistationarity are $K_{12} = K_{21} = 1$ and $K_{22} = 2$. With these parameters, the classical state table is

$x\ y$	$X\ Y$
01	10
01	00
⓪2	02
10	11
11	01
12	02

We see only one steady state, ⓪2, in this table but we know there are two others: 0²θ and 1¹θ. The complete state table becomes:



This description, of course, fits with that of Chapter 7, but in addition we see the logical equivalent of the focus (¹θ¹θ) and of the saddle point (0²θ) of the continuous description given in Chapter 8.

The above provides a more general view of processes that have been discussed throughout this book.

First, one can check that *a system without any feedback loop has a single, stable steady state*, already detected in our naïve description under the name “logical stable state”, identified now as an “integer” steady state, and corresponding to a stable node in the differential description.

Consider now a system comprising, among other interactions, a feedback loop that involves variables x , y , and z , acting above thresholds, say, ${}^2\theta$, ${}^1\theta$, ${}^2\theta$, respectively. For proper values of parameters, the loop will be efficient and endow the variables in question with multistationarity or homeostasis according to whether it is positive or negative. *In either case, the criterion of the efficiency of the loop will be the presence of a steady state at the location $x = {}^2\theta$, $y = {}^1\theta$, $z = {}^2\theta$ in the subspace of the variables involved in the loop.*

When a system comprises two or more feedback loops that have no variables in common, one must also consider a steady state located at the relevant thresholds of all the variables involved. For example, in the system

$$\begin{pmatrix} \ominus 1 & +1 \\ 0 & \ominus 2 \end{pmatrix}$$

there is a negative feedback loop on variable 1x and one on variable 2y . According to the parameter values, there will be a steady state $^1\theta$ (imposed by the first loop), $^{-2}\theta$ (imposed by the second loop), or $^1\theta^2\theta$, "between" the two loops (00 if neither loop is efficient).

One can now ask in a more general way how one can tell whether a state is steady. Consider a state in which n of the variables are located on thresholds. One can convince oneself that this state is not steady unless the variables in question form an n -element loop (with each variable acting at the threshold considered), or else they form two or more loops that do not share any variables. If, however, one of these conditions is fulfilled, the state can be steady, provided the parameter values meet well-defined constraints, as follows. A state located on n thresholds is at the junction of 2^n integer states. Among the transitions between these states, special attention must be paid to those transitions generated by the loop considered. For each of these transitions, the jump of the relevant function must encompass the corresponding threshold of the variable.

For example, the in the system

$$\begin{pmatrix} 0 & -1 \\ +1 & +1 \end{pmatrix}$$

(last cases in Chapter 7), state $^1\theta^1\theta$ could be steady because variables x and y form a feedback loop involving these thresholds. Are there parameter values for which $^1\theta^1\theta$ is indeed steady? The state table is

xy	X	Y
00	K_{12}	0
01	0	K_{22}
10	K_{12}	K_{21}
11	0	$K_{21} + 22$

We see that the value of function Y can change as a result of a transition of either x or y . However, as regards the loop that may generate a steady state at $^1\theta^1\theta$, the relevant change is the crossing of its threshold value by variable x : transitions $00 \rightarrow 10$ (in which Y jumps from 0 to K_{21}), and $01 \rightarrow 11$ (in which Y jumps from K_{22} to $K_{21} + 22$). The conditions for $^1\theta^1\theta$ being steady are

1. That the jump of function X from K_{12} to 0 encompasses the threshold value of variable x ; thus, $^1\theta \in [0, K_{12}]$. (In practice, $K_{12} = 1$.)
2. That the jumps of function Y from 0 to K_{21} and from K_{22} to $K_{21} + 22$ both encompass the threshold of variable y ; i.e., $^1\theta \in [0, K_{21}]$ and $^1\theta \in [K_{22}, K_{21} + 22]$. (In practice, one needs $K_{21} = 1$; $K_{22} = 0$; $K_{21} + 22 = 1$.)

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Part II: Feedback Loops



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I. THE NOTION OF FEEDBACK

Stable entities such as cells, organs, organisms, societies, and sufficiently complex machines must constantly receive information not only about the external environment (via input variables), but also about the state of some of their own elements. The information is then used to make suitable adjustments (via internal functions). This is called *feedback*, or retroaction, operating on internal functions. The basic principle is that any deviation Δx in the value of a variable x triggers a readjustment of x itself. An element can affect its own rate of synthesis (or evolution) either directly or via a chain of interactions with other elements; e.g., substance x may affect the evolution of y , which affects that of z , which in turn affects that of x . Elements connected by a closed chain of interactions of this type form a *feedback loop*. It is clear that each element of a loop affects its own rate of synthesis indirectly via the chain of interactions; this is feedback proper. The elements of a *simple feedback loop* are not subject to other interactions; each element is directly affected only by its immediate predecessor.

II. SIMPLE FEEDBACK LOOPS, POSITIVE OR NEGATIVE

A simple n -element feedback loop involves n interactions, each of which can be positive or negative. The number of different loops possible thus grows very rapidly with the number of elements. However, a major simplification results from the important observation that in any simple feedback loop, either all elements exert a positive effect on their own evolution or all exert a negative effect on their own evolution. ***There are thus two basic classes of simple feedback loops: positive and negative.***

This fundamental fact, although intuitively obvious to some people, is so important that we will look at it a little more closely to see why it is so. Let us first examine a simple feedback loop in which all interactions are positive. Here, each element stimulates the evolution of its follower and hence, indirectly, of itself, whatever the number of elements. An increase (or decrease) in the concentration of any element will stimulate (or depress) the rate of synthesis of its follower, and this effect will work its way around the loop, ultimately reaching the original element. Thus, each element exerts a positive effect on its own evolution: an increase stimulates its evolution and a decrease depresses it.

Let us now imagine a simple n -element loop with a single negative interaction (and $n - 1$ positive interactions). Here, an increase in the concentration of any element will stimulate the evolution of its followers until the negative interaction is reached. The increased concentration of the negatively acting element will depress the rate of synthesis of its follower, and this effect will then be transmitted, via the remaining positive interactions, to the original element, depressing its rate of synthesis. Similarly, a decrease in the concentration of any element will depress the evolution of its followers up to the negative interaction, at which point the effect becomes stimulating (*less of a negative regulator stimulates the rate of synthesis of the following element*). Thus, each element of this loop exerts a negative effect on its own evolution: an increase depresses its synthesis and a decrease stimulates it.

The principle is now clear: each negative interaction in a loop reverses the effect of a perturbation; increases become decreases and vice versa. Thus, the effect an element exerts on itself will be positive if the number of negative interactions in the loop is even and negative if it is odd. It is also clear why each element of a given loop must have the same effect (positive or negative) on its own evolution: it is because this effect results from going around the entire chain of interactions. The starting point is immaterial; what counts is the number of negative interactions, each of which reverses the effect of a perturbation.

We can summarize the above remarks as follows. ***A simple feedback loop is posi-***

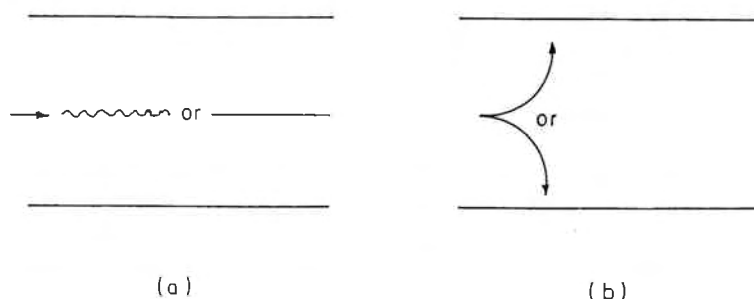


FIGURE 1. Homeostatic (a) and epigenetic (b) regulation. The arrows indicate the evolution of the regulated variable.

tive or negative according to whether it contains an even or odd number of negative interactions. In a simple positive loop, each element exerts a positive effect on its own rate of synthesis, whereas in a simple negative loop, each element exerts a negative effect on its own rate of synthesis.

It is interesting to note that in a formal sense, all positive loops are equivalent to each other and all negative loops are equivalent to each other, at least in terms of the effect an element exerts on its own synthesis. Intuitively, it is clear that a chain of positive interactions is formally equivalent to a simple direct positive interaction between the two extreme elements, and that any two negative interactions cancel out. In this way, any simple feedback loop can be reduced, on paper at least, to a one-element loop, positive or negative according to the number of negative interactions (even or odd) in the original loop. In actual fact, however, a simple multielement loop contains more information than just its parity, positive or negative. Although the sign determines the essential character of the regulation involved, as discussed below, loops of different length can express this behavior in different ways.

III. THE BEHAVIOR OF POSITIVE AND NEGATIVE FEEDBACK LOOPS

As briefly mentioned in the Prologue, there are two basic types of regulation that give rise to diametrically opposite patterns of behavior: *homeostatic regulation* tends to maintain a variable near a specific (supposedly optimal) value and *epigenetic* (or differentiative) *regulation* forces the system to choose between the extreme values of a variable and to keep the variable permanently at one or the other level (Figure 1). These two types of regulation are mediated by simple feedback loops: homeostasis by negative loops and differentiation by positive loops. This is probably already clear to the reader, who will have noticed that we deliberately chose simple feedback loops as examples in Part I. We will illustrate these two behavioral patterns here with concrete examples.

A. THE NEGATIVE LOOP

An example of a biological system that behaves as a simple negative feedback loop is a product \underline{x} which inhibits its own synthesis. This type of regulation is observed for many metabolites such as amino acids. At low concentrations of \underline{x} , synthesis will be uninhibited and the pool of \underline{x} will build up. At high concentrations, on the other hand, synthesis will be blocked. Under these conditions, the concentration will drop for any of several reasons: the cell may be using \underline{x} (e.g., incorporating it into proteins or other macromolecules) or excreting it, \underline{x} may be metabolically unstable (e.g., a precursor of another metabolite), and in any case \underline{x} will be diluted out by growth. When the concentration of \underline{x} has dropped sufficiently, synthesis will no longer be efficiently inhibited. Thus, the concentration of \underline{x} can be

expected to oscillate around or approach a certain fixed value, intermediate between the extreme (unregulated) values that would be obtained if synthesis were permanently inhibited or never inhibited; this is *homeostasis*. Since, as discussed above, all simple negative feedback loops are formally equivalent in the sense that any element in such a loop exerts a negative effect on its own rate of synthesis, we conclude that **simple negative feedback loops result in homeostasis**. This point is treated in detail in Chapters 10 and 11.

In real experimental conditions, oscillation is not usually observed in one-element systems. As discussed in Chapter 6, synthesis tends to stabilize at a rate equal to the rate of decay or dissipation. With more elements or with appropriate time delays, oscillations can, in fact, occur, either damped or permanent.

Metabolite pools, to come back to our example, are normally maintained at a fixed level in a given environment. A number of different mechanisms have been discovered for bringing this about, but all are based on a negative feedback loop. One, called *end-product inhibition*, involves direct inhibition, by the final metabolite \underline{x} , of an enzyme involved in the biosynthesis of \underline{x} . This is usually the enzyme that catalyzes the first reaction specific to synthesis of \underline{x} , thus avoiding accumulation of intermediates.¹ End-product inhibition is a highly efficient, rapidly acting homeostatic mechanism. A second mechanism is end-product *repression* of enzyme synthesis. In many biosynthetic pathways, the relevant genes are regulated by a repressor which is only active when the concentration of the final metabolite is high.² This mechanism is a useful energy saving device for the cell since it turns off the synthesis of unneeded enzymes. A third mechanism, discovered by Yanofsky and co-workers,³ is called *attenuation*. A typical example, in fact the first discovered, is that of the genes involved in tryptophan biosynthesis. These genes form an operon, transcribed in a single mRNA molecule. When the tryptophan pool is normal, transcription starts at the usual site, but is efficiently terminated 140 nucleotides away, before any of the relevant genes have been transcribed. This termination requires a particular configuration of the short mRNA molecule, which can only occur if the mRNA is rapidly translated, i.e., if the ribosomes are close behind the RNA polymerase. If translation is slow, the mRNA takes on a second configuration which prevents the transcriptional stop signal from forming, and the entire operon is then transcribed. The short mRNA codes for a "leader peptide" of 14 amino acids, containing two adjacent tryptophan residues. Its translation speed is therefore very sensitive to the tryptophan concentration: if tryptophan is limiting, the leader peptide will be translated slowly and the mRNA will assume the nontermination configuration, resulting in efficient transcription of the entire operon. Thus a low tryptophan pool rapidly stimulates synthesis of the tryptophan biosynthetic enzymes.

Attenuation, like end-product repression, prevents the synthesis of unneeded enzymes. These two mechanisms are formally equivalent to end-product inhibition and will, in fact, stabilize metabolite pools at a fixed level, but the stabilization can be extremely slow. If, for example, a metabolite (such as tryptophan) suddenly appears in the medium, repression or attenuation will immediately repress the synthesis of the relevant enzyme, but will not inhibit the enzyme molecules already present in the cell that will tend to overproduce the metabolite in question. The enzyme molecules must simply be diluted out by growth, so several generations may be required before the pool size returns to its normal level. End-product inhibition, in contrast, would immediately block the synthesis of the metabolite. Thus, the same logical circuit — a simple negative feedback loop — is carried out by the cell via very different molecular mechanisms to respond to different physiological needs. End-product inhibition maintains a constant metabolite pool, whereas end-product repression and attenuation maintain enzyme levels appropriate to the cell's needs.

B. THE POSITIVE LOOP

An example of a biological system that behaves as a simple positive feedback loop is a gene \underline{X} whose expression requires the presence of its own product \underline{x} . This is the case, for

example, for the cI repressor in λ lysogens⁴ (cf. Chapter 20). If we provisionally neglect the role of two other regulators of cI expression, the analysis is straightforward: either \underline{x} is present, in which case it promotes its further synthesis (via gene \underline{X} expression) and thus remains present, or \underline{x} is absent, gene \underline{X} cannot be expressed, and \underline{x} remains absent. The system is seen to have a choice between two stable steady states in which the variable is maintained at one or the other extreme value. This is *epigenetic* or *differential* regulation.

Again, the formal equivalence of all simple positive feedback loops permits us to conclude that **simple positive feedback loops result in epigenetic regulation, with multiple steady states**. This point is treated in detail in Chapters 12, 13, 14, and 15.

The positive autoregulation of the λ repressor is thought to play an important role in prophage induction. Lysogenic bacteria harbor a prophage whose repressor prevents the expression of other phage genes. If the cell's DNA should become damaged (for example, by ultraviolet radiation), the SOS response will be induced and λ repressor will begin to be degraded by the RecA protein (cf. Chapter 19). As long as the rate of this degradation is minor, repressor synthesis can maintain a sufficient pool of active repressor. If, however, the repressor concentration drops too low to stimulate further synthesis, the cI gene is suddenly turned off and the prophage is quickly derepressed. This makes induction an all-or-none phenomenon. The point at which prophage induction is irreversibly triggered depends on the threshold of repressor autoregulation. In the case of phage λ , it corresponds to a level of DNA damage that normally leaves the bacterium little chance to survive. The induced phage leaving a damaged cell is therefore somewhat like a rat leaving a sinking ship, and positive autoregulation of the repressor lets the phage take a position somewhere between the captain who goes down with the ship (near-zero threshold*), and the nervous passenger who abandons ship as soon as the going gets rough (positive autoregulation with a very high threshold**). In all cases, however, there are two stable steady states, in which the cI gene is permanently on or permanently off.

IV. INDIVIDUAL CONTROLS VS. FEEDBACK LOOPS

It is a common error in discussing biological regulation to confuse individual regulatory controls (which may be positive or negative) and feedback loops (which may also be positive or negative). Feedback loops consist of circular series of interactions, which may be all positive, all negative, or mixed. In particular, a positive loop may include (an even number of) negative interactions and a negative loop can contain any number of positive interactions. On the other hand, biological regulation, brought about by positive or negative interactions between elements, does not necessarily form a feedback loop. For example, the RecA protein is a negative regulator of the λ repressor — it degrades the repressor after ultraviolet irradiation (cf. Chapter 19). Although it is a fundamental regulation and the basis of lysogenic induction, it clearly does not constitute a feedback loop since the λ repressor does not regulate expression of the RecA protein.

When one of us (R. T.) discovered positive control of gene expression, the difference between positive and negative regulation seemed fundamental.⁵ Today, we feel that the real essence of a regulatory circuit is not whether any individual control step is positive or negative, but rather whether the feedback loops involved are positive or negative. In fact, although positive regulators are common in nature, one could, in principle, construct all control networks using only negative interactions. With only positive regulatory interactions, however, circuits would be limited to positive loops and would be quite incapable of accounting for most biological behavior.

* Or essentially no autoregulation: repressor can be made even in the face of massive repressor degradation.

** Repressor synthesis is turned off after even a slight drop in concentration.

To clarify these notions, let us consider the lactose operon, which was the first system of biological regulation to be understood through the impressive work of Jacob and Monod.⁶ It was known from the pioneering work of Monod, Cohn, and co-workers that the bacterium *Escherichia coli* does not synthesize the proteins required for lactose utilization unless there is lactose in the medium. Jacob and Monod showed that this is brought about by a negative regulator, the *lac* repressor, which prevents the expression of the relevant genes in the absence of lactose. In the presence of lactose, the repressor is inactivated (in fact, by allolactose, a simple derivative of lactose), the *lac* genes are expressed, and lactose can be catabolized. This regulatory scheme, which had a tremendous impact on modern biological thinking, involves a negative interaction — that of the repressor with the *lac* genes — but is not a feedback loop, positive or negative. The system can, in fact, be included in a feedback loop simply by considering another interaction. Lactose must enter the cell to lead to induction, and at low exogenous lactose concentrations, diffusion is insufficient. Entry therefore requires the lactose permease, which is only synthesized in the presence of lactose. This typical vicious circle results from a *positive* feedback loop: permease is synthesized only if lactose can get into the cell, but entry requires permease. This example is discussed in Chapter 17.

V. KEY TO PART II

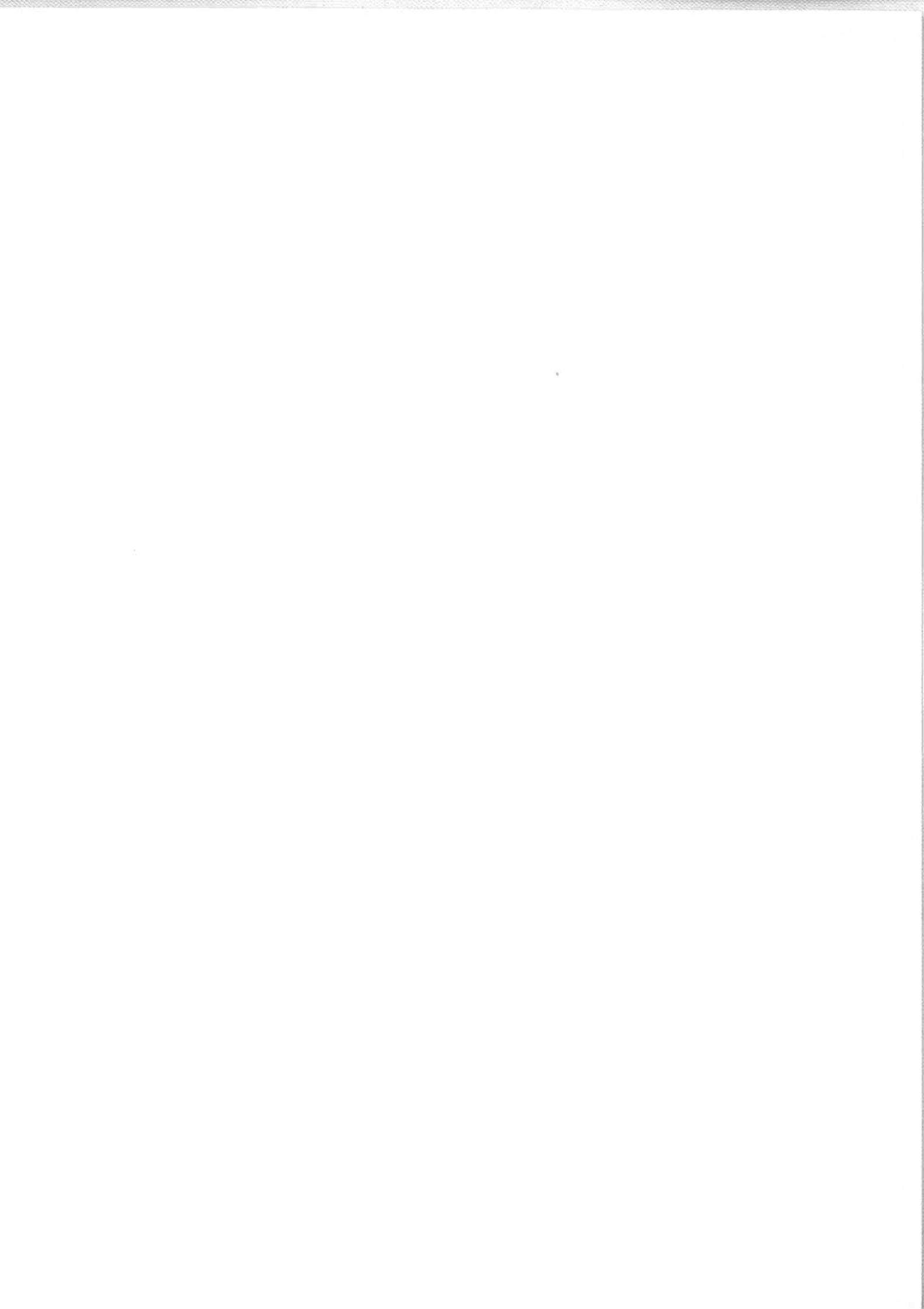
In Part II, we are concerned with feedback loops: what they are, how they work, and what patterns of behavior they can produce. We first consider simple feedback loops, whose constituent elements are independent of the other internal variables in the system. We show how simple negative loops generate homeostasis, with or without oscillation (Chapters 10 and 11) and how simple positive loops generate multistationarity, i.e., differentiation (Chapters 12 and 13). In these chapters, we present the logical (Boolean) analysis and the differential description of feedback loops. For both positive and negative loops, we begin with the differential descriptions (Chapters 10 and 12), despite the fact that (1) our approach has involved the development of a logical method and (2) most biologists find the differential description more difficult. We chose this order because biologists tend *a priori* to have more confidence in the differential description, despite its heaviness. Basically, they consider the differential description *reality* and the logical description its *caricature*. Indeed, we ourselves originally thought this! In actual fact, this attitude is inaccurate for several reasons. First of all, experience has shown that there is usually a broad domain of parameter values for which the two descriptions agree qualitatively, so the essential behavioral patterns of a continuous system can be deduced from the much easier analysis of its discrete Boolean counterpart. Second, although in the differential description the value of each variable can be calculated for each point in time to as many decimal places as we wish, this is only an illusion of accuracy: in fact, for each interaction in the system, the description includes a precise mathematical curve (usually a sigmoid) whose exact shape and parameters are often quite arbitrary. However, the elegant differential equations, with all the power and prestige of quantitative analysis, tend to make one forget their arbitrary aspects, whereas it would be difficult to forget that step functions are idealizations. Finally, the differential system almost invariably neglects the existence of absolute, incompressible time delays (such as those required for the transcription and translation of a gene after it has been turned on). Actually, certain differences between the discrete and differential descriptions vanish if time delays are introduced into the latter; e.g., one- and two-element negative loops can exhibit sustained oscillations (cf. Richelle⁷).

In the remainder of Part II, we examine systems which include more than a single feedback loop to see how more complex patterns of behavior can be generated. In Chapter 14, we show how a transient signal can result in a lasting change of behavior; in Chapter 15, we see how one can easily obtain *many* steady states; and in Chapter 16, we illustrate some elemen-

tary effects produced by combining positive and negative feedback loops in more complex circuits. We hope that some readers at least will be amazed at the rich variety of behavior that can result from relatively simple logical circuits.

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SIMPLE NEGATIVE FEEDBACK LOOPS GENERATE HOMEOSTASIS: DIFFERENTIAL DESCRIPTION

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I. INTRODUCTION

Simple negative feedback loops generate homeostasis, as discussed in the preceding chapter. A simple loop is *negative* if it comprises an *odd* number of negative interactions. In this chapter, we will consider sigmoid interactions or their limit case, the step function. In actual fact, much of what is said about sigmoid functions can be extended to other *monotonic* functions. For instance, *some* of the interactions of a loop can be linear without changing the qualitative picture.

Homeostasis can be illustrated by the Watt regulator, by natural or man-made thermostats, or by biological regulatory mechanisms that block further synthesis of a metabolite when its concentration is already high. In such systems, an element exerts a negative retroaction on its *own* further evolution, i.e., all are based on a negative feedback loop. In Chapter 9, we showed that simple negative loops generate homeostasis. In the present chapter, using the differential description, we analyze *how* they do this: under what conditions and to what extent homeostasis is actually obtained and when it leads to stable periodic behavior. (See References 1 to 5.)

II. DIFFERENTIAL DESCRIPTION OF SIMPLE NEGATIVE LOOPS

A. THE ONE-ELEMENT NEGATIVE LOOP

1. General

The simplest negative loop consists of a single element x , which exerts a negative effect on its own synthesis ($x \overset{\curvearrowright}{\rightarrow}$). The higher the *concentration* of x , the lower its *rate of synthesis*.

There are many genes whose expression is repressed by their own product. Strictly speaking, this is usually not a true one-element negative loop since most gene products are proteins manufactured by an mRNA intermediate and it is often the latter whose synthesis is regulated. In this book, we shall usually treat gene expression as a whole, but one can, of course, explicitly formalize transcription and translation if necessary. In any case, treating the one-element negative loop is useful, if only because it is the simplest possible case.

Our system can be described as follows:

$$\frac{dx}{dt} = H(x) = kF^-(x) - k_-x \quad (1)$$

in which, as usual, F^- is a decreasing Hill function and k and k_- are positive kinetic constants related to the rates of synthesis and decay of substance x , respectively. We consider only nonnegative values of x because it is a concentration. It can be seen from Equation (1) that $\frac{dx}{dt}$ comprises a positive term (synthesis), $kF^-(x)$, which equals k for $x = 0$ and monotonically approaches 0 for high values of x , and a negative term (decay), which is nil for $x = 0$ and increases proportionally to x . Thus (see Figure 1A, in which $\frac{dx}{dt}$ is plotted as a function of x), for low values of x , the time derivative $\frac{dx}{dt}$ is positive and x increases, whereas for high values of x , it is negative and x decreases.

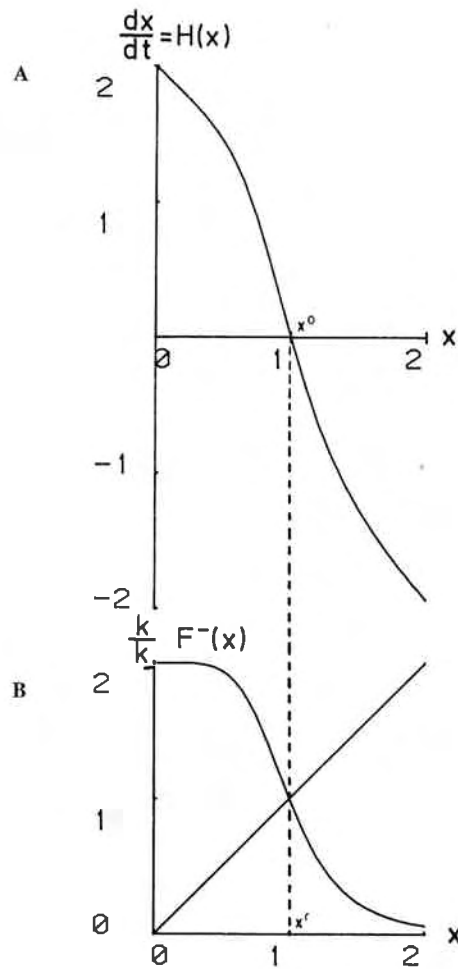


FIGURE 1. One-element negative loop: $\frac{dx}{dt} = H(x) = kF^-(x) - k_-x$. (A) The time derivative $H(x)$ as a function of x . The intersect of the curve with the x axis is the steady-state value x^0 . In our particular case, $H(x) = \frac{2}{1+x^5} - x$. (B) The steady-state equation $\frac{dx}{dt} = 0$ or $kF^-(x) - k_-x = 0$ can be written $x = \frac{k}{k_-} F^-(x)$, or $x = G^-(x)$. If, instead of plotting $H(x)$ as a function of x , one plots $G^-(x)$, the steady state will be given by the intersect between $G^-(x)$ and the bissectrix. In the particular case given here, the steady-state equation is $\frac{2}{1+x^5} - x = 0$, or $x = G^-(x) = \frac{2}{1+x^5}$. The steady-state value (the solution of the steady-state equation), $x^0 = 1$, is readily found by iteration (see Appendix 1).

2. The Unique Steady State of the One-Element Negative Loop

When the system is at steady state, we have $\frac{dx}{dt} = H(x) = 0$, and the system will remain as it is, in the absence of external change. In our case, the steady-state equation of the system is

$$kF^-(x) - k_-x = 0 \quad (2)$$

The solution(s) can be visualized by plotting $H(x)$ as a function of x and looking at the intersection(s) with the x axis (Figure 1A). It is often more convenient, however, to rewrite the

steady-state Equation (2) in the form:

$$x = \frac{k}{k_-} F^-(x) \quad (3)$$

The solutions are those values of x for which $\frac{k}{k_-} F^-(x)$ and x itself are equal, in other words, the values of x at the intersects between the graphs $\frac{k}{k_-} F^-(x)$ and x (which is the bisectrix of the positive quadrant; see Figure 1B). Since $\frac{k}{k_-} F^-(x)$ is a positive, monotonically decreasing function of x , it will have exactly one intersect with the bisectrix. *Our negative loop thus has only one steady state.*

The algebraic Equation (3) cannot be solved analytically for $n > 3$, where n is the value of the exponent in the Hill function $F^-(x)$. As a first step, we have solved it graphically (see Figure 1A and B).

Fortunately, for any given values of the parameters $\frac{k}{k_-}$, θ , and n , the steady state can be calculated using an appropriate iterative (numerical) method (see Appendix 1).

As shown in Appendix 3 and Chapter 6, for a one-variable system in general, a small perturbation will regress or amplify (and, accordingly, the steady state will be stable or unstable), depending simply on whether $\frac{dH(x^0)}{dx}$ is negative or positive, in other words, on whether the slope of $H(x)$ at x^0 is negative or positive.

In the case of the *one-element negative loop*, $H(x)$ is monotonically decreasing, with $H(0) > 0$. This implies that (1) as noted above, there is a *single steady state* and (2) since the slope $\frac{dH}{dx}$ is negative everywhere, and in particular at x^0 , the unique steady state x^0 is stable.

3. The Domain in Which a One-Element Negative Loop Effectively Promotes Homeostasis

At this point, we would like to consider the steady-state value in the extreme case of a very steep sigmoid ($n \rightarrow \infty$ in the Hill function). We thus have

$$\frac{k}{k_-} F^-(x, \theta) \approx 0 \text{ for } x > \theta$$

$$\frac{k}{k_-} F^-(x, \theta) \approx \frac{k}{k_-} \text{ for } x < \theta$$

The steady state is located at the intersect of this curve with the bisectrix $y = x$. Clearly, there are two qualitatively different situations, according to whether $k/k_- > \theta$ or $k/k_- < \theta$. The steady-state value in the first case is $x^0 \approx \theta$ and in the second, $x^0 \approx k/k_-$ (Figure 2a and b).

The interest of this observation can be seen by the following reasoning. A steady-state value $x^0 = k/k_-$ is the same as that of an *unregulated* system in which product \underline{x} is permanently synthesized at a constant rate k independently of its concentration (i.e., $dx/dt = k - k_-x$, which has the steady-state value $x^0 = k/k_-$). Thus, for $k/k_- < \theta$, the system is essentially unregulated and there is no homeostasis. The *homeostatic effect* of the negative loop, which makes the rate of synthesis of \underline{x} dependent on its own concentration and provides a steady-state value significantly lower than k/k_- , is only effective in the domain of parameters $k/k_- > \theta$. In this case, the steady-state value of \underline{x} is close to θ itself (the higher n , the closer it will be). In the case of a thermostat, this simply means that it is impossible to regulate the

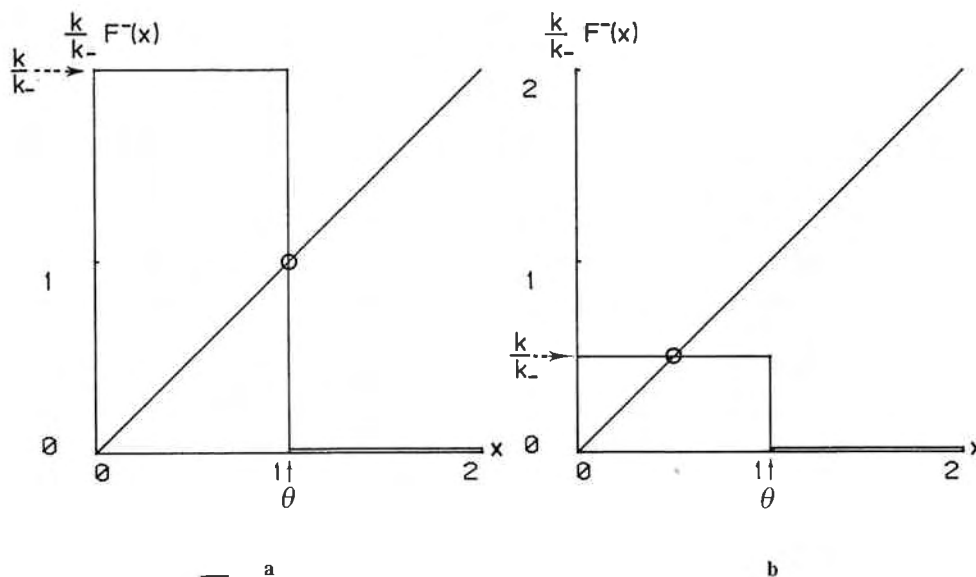


FIGURE 2. When does a one-element loop effectively promote homeostasis? Plot of $\frac{k}{k_-} F(x, \theta)$ as a function of x , for an extremely steep sigmoid ($n \rightarrow \infty$). As usual, the steady state x^0 is given by the intersect of this curve with the bisectrix.

(a) $k/k_- = 2.0$, $\theta = 1$.

(b) $k/k_- = 0.5$, $\theta = 1$.

In the first case, $k/k_- > \theta$, the steady-state value is $\approx \theta$ and homeostasis is effective. In the second case, $k/k_- < \theta$ and the steady-state value is k/k_- , as if there were no regulation.

system to a temperature θ higher than that obtained by leaving the heater permanently in the "on" position!

To the extent that homeostasis is the "purpose" of negative loops, one might say that (for high values of n) our negative loop is *effective* only for $\frac{k}{k_-} > \theta$ and that when this is the case, the steady-state value is close to θ . For lower values of n , the statement should be expressed more cautiously: the loop is effective only if $\frac{k}{k_-}$ is "sufficiently higher" than θ .^{*} As we shall see, this type of fuzzy statement can be very useful.

4. Trajectory and Evolution of a One-Element Negative Loop

In the present case, we are dealing with a one-variable system, so the variable space is simply the nonnegative half of the x axis. On this axis, there is a single, stable steady state, x^0 .

From any initial state to the left of x^0 , the trajectory will be rightward (increasing x) toward x^0 , and from any initial state to the right of x^0 , the system will proceed leftward (decreasing x) toward x^0 (Figure 3a).

The evolution of a system is a description of its state as a function of time (here, the value of x as a function of time). Whatever the initial state, the present system proceeds asymptotically toward the unique, stable steady state (Figure 3b). As we will see elsewhere

* More precisely, to have a steady state value less than $(1 - \epsilon) \frac{k}{k_-}$, we must have $\frac{k}{k_-} > \theta \cdot \frac{1}{1 - \epsilon} \left(\frac{\epsilon}{1 - \epsilon} \right)^{\frac{1}{n}}$

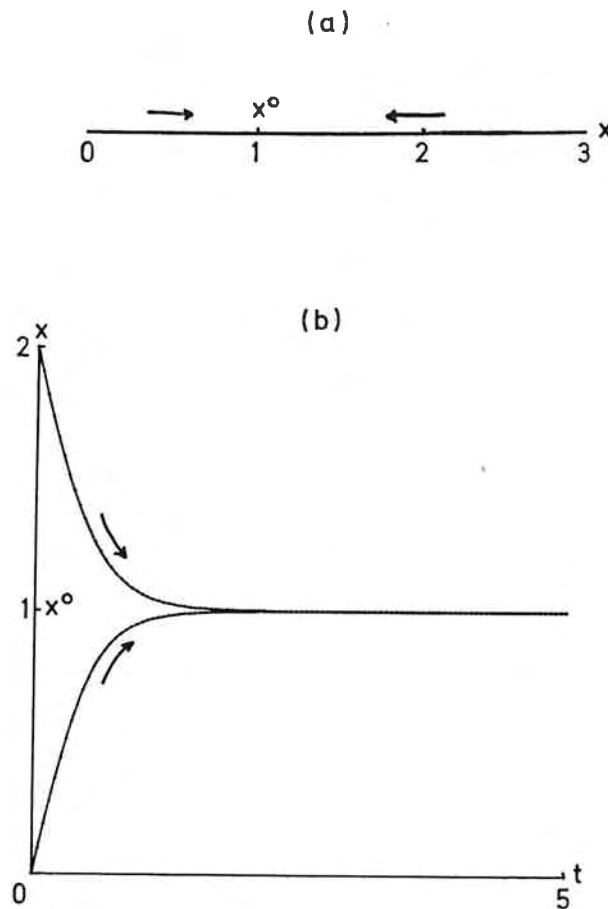


FIGURE 3. One-element negative loop: trajectories and evolution. Same parameters as in Figure 1. (a) Trajectories; (b) evolution. Variable x is plotted as a function of time for two initial values ($x = 0, x = 2$).

(Chapter 20), one result is that a gene subject to efficient negative autoregulation will be insensitive to the number of gene copies present (gene dosage).

In summary, for a one variable system, a steady state x^0 is stable or unstable, depending simply on whether the slope $\frac{dH(x^0)}{dx}$ is negative or positive. A *one-element negative loop* (at least in a differential description without time delays) *has a single, stable steady state*. The loop will effectively generate homeostasis if $\frac{k}{k_-}$ is sufficiently greater than θ . In this case, the steady-state value will be close to θ itself.

B. THE TWO-ELEMENT NEGATIVE LOOP: DAMPED PERIODIC BEHAVIOR

1. Description and Steady State

The simple two-element negative loop described by the graph $x \begin{array}{c} \xrightarrow{+} y \\ \xleftarrow{-} x \end{array}$ and the equations

$$\begin{aligned}\frac{dx}{dt} &= H_x = k_1 F^-(y, \theta_y) - k_{-1}x \\ \frac{dy}{dt} &= H_y = k_2 F^+(x, \theta_x) - k_{-2}y\end{aligned}\quad (4)$$

were analyzed in Chapter 6 (Sections V — VIII). There, we insisted more on the *methodology* than on the *properties* of the loop. We saw that such a loop always has a single steady state. We will now show that this steady state is always stable and will analyze the approach to it. As described in Appendix 3, the role of the derivative is played here by the Jacobian matrix:

$$\begin{bmatrix} \frac{\partial H_x}{\partial x} & \frac{\partial H_x}{\partial y} \\ \frac{\partial H_y}{\partial x} & \frac{\partial H_y}{\partial y} \end{bmatrix} = \begin{bmatrix} -k_{-1} & k_1 \frac{dF^-(y)}{dy} \\ k_2 \frac{dF^+(x)}{dx} & -k_{-2} \end{bmatrix}$$

and the stability properties of a steady state depend on the roots of the characteristic equation:

$$\begin{vmatrix} a_{11} - \omega & a_{12} \\ a_{21} & a_{22} - \omega \end{vmatrix} = 0$$

in which the terms a_{ij} are the corresponding terms of the Jacobian matrix evaluated at the steady state considered. Expanding the determinant gives the characteristic equation:

$$\omega^2 - (a_{11} + a_{22})\omega + a_{11}a_{22} - a_{12}a_{21} = 0.$$

A quadratic equation can be written $\omega^2 - S\omega + P = 0$, in which S is the sum and P the product of the roots. We thus have $S = (a_{11} + a_{22})$ and $P = a_{11}a_{22} - a_{12}a_{21}$.

The stability of the single steady state depends on the signs of the roots of the characteristic equation, or on the sign of the real part if the two roots are complex conjugates (Appendix 3). The sum of the roots is given by $S = a_{11} + a_{22} = -k_{-1} - k_{-2} < 0$, so in the case of complex roots the real part, which equals half the sum, is negative and the steady state will be a stable focus, approached periodically. In the expression for the product of the roots, $P = a_{11}a_{22} - a_{12}a_{21}$, the first term, which is the product of the positive kinetic constants k_{-1} and k_{-2} , must be positive; the second term, $-a_{12}a_{21}$, must also be positive since the derivatives of $F^+(x)$ and $F^-(y)$ have opposite signs (positive and negative, respectively). Thus, the product of the roots is positive. Therefore, if the two roots are real, they must both be negative and the steady state will be a stable node, approached directly. In all cases, *the two-element simple negative loop has a stable steady state*.

What determines whether this steady state is a focus (complex roots) or a node (real roots)? The roots of a quadratic equation are real or complex conjugates according to whether $S^2 - 4P \geq 0$ or < 0 . Here,

$$\begin{aligned}S^2 - 4P &= (a_{11} + a_{22})^2 - 4a_{11}a_{22} + 4a_{12}a_{21} \\ &= (a_{11} - a_{22})^2 + 4a_{12}a_{21}\end{aligned}$$

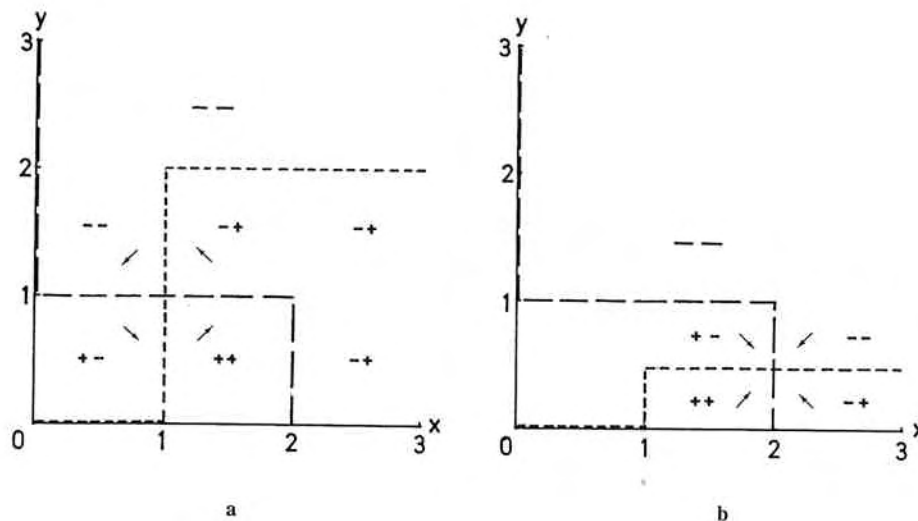


FIGURE 4. When does a two-element negative loop effectively promote homeostasis? The nullclines of System 4 in the case of extremely steep sigmoid interactions ($n \rightarrow \infty$): (a) $k_1 = 2$, $k_2 = 2$, $k_{-1} = 1$, $k_{-2} = 1$, the θ s = 1; (b) same, but $k_{-2} = 4$. In the first case, $k_1/k_{-1} > \theta_x$, and $k_2/k_{-2} > \theta_y$, the steady state is located near (θ_x, θ_y) and homeostasis is effective. In the second case, one of the conditions is not fulfilled ($k_2/k_{-2} < \theta_y$); the steady state is at $(k_1/k_{-1}, k_2/k_{-2})$.

The second term is negative since a_{12} and a_{21} have opposite signs in a negative loop. The first term is positive or zero, depending on whether a_{11} and a_{22} — that is, k_{-1} and k_{-2} — are different or not.

An inescapable conclusion is that for equal values of k_{-1} and k_{-2} , $S^2 - 4P$ is necessarily negative and we have a focus. More generally, one might be tempted to answer the above question by simply saying that $S^2 - 4P$ can be positive (and the steady state a node) only if $|k_{-1} - k_{-2}|$ is sufficiently large. However, this is not the whole story, as we will see now.

2. Under What Conditions Will Homeostasis Be Effective?

Let us consider a simple two-element negative loop with extremely steep sigmoid interactions, using two different sets of parameter values (Figure 4a and b).

In the first case, the steady state is located at (θ_x, θ_y) . Both variables approach a value well above the off value (0, 0) and well below the unregulated value $(\frac{k_1}{k_{-1}}, \frac{k_2}{k_{-2}})$. Homeostasis is thus achieved.

In the second case, the steady state is located at $(\frac{k_1}{k_{-1}}, \frac{k_2}{k_{-2}})$, which corresponds to the unregulated value. Homeostasis is *not* achieved.

It is easy to see that (for extremely steep sigmoids) the conditions for the first situation are simply $\frac{k_1}{k_{-1}} > \theta_x$ and $\frac{k_2}{k_{-2}} > \theta_y$. If either of these conditions is not fulfilled, the steady-state value will be $(k_1/k_{-1}, 0)$ or $(k_1/k_{-1}, k_2/k_{-2})$ rather than (θ_x, θ_y) , and homeostasis will not occur.

Another way to attack this problem is to consider the equation $x = G_1^{-1}(G_2^+(x))$, derived from the steady-state equations of the system (cf. Chapter 6, Section VI). One sees immediately (Figure 5a and b) that, according to the parameter values, the intersect between graphs $G_1^{-1}(G_2^+(x))$ and x , which provides the steady-state value of variable x , is on $(x = \theta)$ or on $(x = k_1/k_{-1})$.

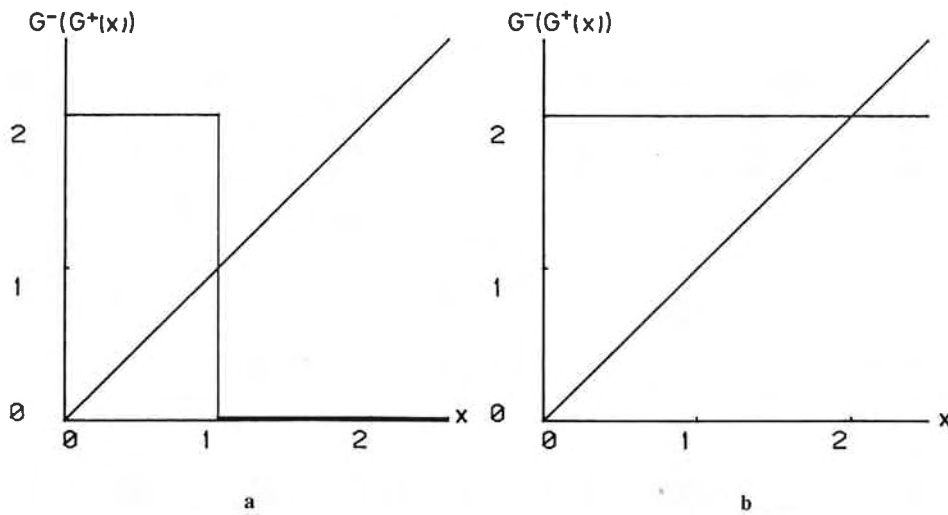


FIGURE 5. Same situation as given in Figure 4, but here we plot $G_1(G_2^*(x))$ as a function of x , and the steady-state value of variable x is given by the intersect of this curve with the bisectrix.

Note that in the first case:

$$\text{for } x < \theta \quad G_x = k_1/k_{-1}$$

$$\text{for } x > \theta \quad G_x = 0$$

$$\text{for } x = \theta \quad G_x \in \left[0, \frac{k_1}{k_{-1}} \right]$$

using the nomenclature of Chapter 8, Section V.

Thus, for sufficiently steep interactions, a simple two-element negative loop ensures effective homeostasis, provided both conditions, $k_1/k_{-1} > \theta_x$ and $k_2/k_{-2} > \theta_y$ are fulfilled. For less steep interactions, the conditions are that k_1/k_{-1} and k_2/k_{-2} be "sufficiently" higher than θ_x and θ_y , respectively. Once again, as for the one-element negative loop, these conditions simply mean that for homeostasis to be effective, the maximal (fully "on") steady value of each variable must be greater than its threshold value. This is intuitively obvious since, if this threshold cannot be reached, for steep sigmoids the substance is essentially absent in terms of its regulatory ability.

We should now come back to the question already raised above: what determines whether the steady state will be a focus or a node? For this, let us first go back to Figure 4. The signs of the derivatives are indicated ($++$, $+-$, etc.). The arrows show the general direction in which the system will evolve (according to the signs of dx/dt and dy/dt). A look at them suggests that in the first case, in which the conditions $\frac{k}{k_-} > 0$ are fulfilled (Figure 4a), the steady state is approached periodically and is thus a focus, whereas in the second case, in which one of the conditions is not fulfilled (Figure 4b), it is approached directly and is a node. This is confirmed by linear stability analysis.

At this point, one might be tempted to generalize as follows: when the boundary values k/k_- are sufficiently higher than the corresponding threshold values θ , (1) the steady state is near (θ_x, θ_y) , (2) homeostasis is thus ensured, and (3) the steady state is a (stable) focus. If the conditions are not all fulfilled, the steady state is closer to the boundaries, homeostasis is not ensured, and the steady state is a (stable) node.

However, for finite steepness of the interactions, the situation is not so clear-cut: one can find small domains of the parameter values such that, in spite of the fact that the boundary threshold inequalities are fulfilled, the second steady state is a node, or such that, in spite of the unfulfilled conditions, the steady state is a focus. These exceptions are precisely linked to the aspects discussed at the end of Section II.B.1: if the conditions are fulfilled, the steady state is a focus unless the value of $(k_{-1} - k_{-2})^2$ is sufficient to compensate for the negative value of $4a_{12}a_{21}$ and, conversely, if the conditions are not fulfilled, the steady state is a node unless the k_{-} are equal or almost equal (in which case $(k_{-1} - k_{-2})^2$ cannot compensate for the negative value of $4a_{12}a_{21}$). In fact, these "exceptions" deal with very small domains of the parameter space and represent very unusual situations indeed, such as, for example, an almost exact identity of values of the k_{-} . Furthermore, when the steady state is a focus only because $k_{-1} = k_{-2}$, the imaginary part of the root is so small that the periodic character is barely distinguishable.

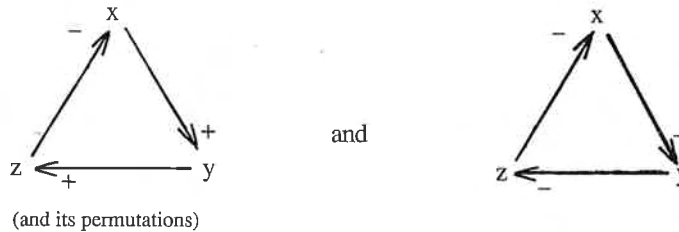
In summary, in continuous systems, a simple two-element negative loop has a *single steady state* that is *stable*. The steady state is located near the threshold values (θ_x, θ_y) (and, consequently, homeostasis is effective), provided the $\frac{k}{k_{-}}$ s are sufficiently higher than the respective θ s. Except for very peculiar situations, when these conditions are fulfilled, the (stable) state is a focus; otherwise it is a node.

C. THREE-ELEMENT NEGATIVE FEEDBACK LOOPS: STABLE PERIODIC BEHAVIOR

1. General

One might question the need for successively describing loops comprising one, two, and three elements. Why not describe the simplest one and simply say: "and the same, *mutatis mutandis*, for loops with more elements"? In fact, we have already seen that proceeding from a one- to a two-element negative loop leads to an enrichment in that the steady state (which is necessarily stable in both cases) may be reached directly or periodically for the latter, but only directly for the former. *As we shall see, with a three-element loop, the steady state may become unstable and generate sustained periodic behavior.*

There are two types of three-element negative loops:



For the first type described above, the differential equations are

$$\begin{aligned}\frac{dx}{dt} &= k_1 F_1^-(z) - k_{-1}x \\ \frac{dy}{dt} &= k_2 F_2^+(x) - k_{-2}y \\ \frac{dz}{dt} &= k_3 F_3^-(y) - k_{-3}z\end{aligned}\tag{5}$$

in which F^- and F^+ are monotonically decreasing and increasing Hill functions, respectively.

However (see below), some of the interactions may be linear instead of sigmoid. The steady-state system, defined by $dx/dt = 0$, $dy/dt = 0$, and $dz/dt = 0$, is

$$x = \frac{k_1}{k_{-1}} F_1^-(z) = G_1^-(z)$$

$$y = \frac{k_2}{k_{-2}} F_2^+(x) = G_2^+(y)$$

$$z = \frac{k_3}{k_{-3}} F_3^+(y) = G_3^+(z)$$

As in Chapter 6, one can write

$$x = G_1^-(G_3^+(G_2^+(x))) = G_x(x)$$

and the resultant function G_x will be a *decreasing* sigmoid because there is an *odd* number of decreasing sigmoids in the chain: $x = G_x(x)$.

As for one- or two-variable systems, the steady-state value of x is given by the intersect between x and $G_x(x)$. Since $G_x(x)$ is a (positive) monotonically decreasing function of x , there is a single intersect and, thus, a *single steady state*.

For any set of parameters values, the values of x^0 , y^0 , and z^0 are easily calculated by an appropriate iteration method (see Appendix 1).

2. Linear Stability Analysis

Here, the Jacobian matrix is

$$\begin{bmatrix} -k_{-1} & 0 & k_1 \frac{dF_1^-(z)}{dz} \\ k_2 \frac{dF_2^+(x)}{dx} & -k_{-2} & 0 \\ 0 & k_3 \frac{dF_3^+(y)}{dy} & -k_{-3} \end{bmatrix}$$

The diagonal terms $-k_i$ correspond to the spontaneous decay rates of the components of the system. The 0 terms reflect the fact that, for instance, x is not directly influenced by the concentration of y . The three terms $k_1 \frac{dF_1^-}{dz}$, etc. describe the looped interactions (dx/dt is directly influenced by z , etc.).

The characteristic equation of the linearized system is

$$\begin{vmatrix} -k_{-1} - \omega & 0 & k_1 \left(\frac{dF_1^-(z)}{dz} \right)_0 \\ k_2 \left(\frac{dF_2^+(x)}{dx} \right)_0 & -k_{-2} - \omega & 0 \\ 0 & k_3 \left(\frac{dF_3^+(y)}{dy} \right)_0 & -k_{-3} - \omega \end{vmatrix} = 0$$

in which the 0 subscripts indicate the values at the steady state.

This is a cubic equation in ω . There are thus three roots, either all real or one real and two complex conjugates. One can easily show that in the case of a three-element negative loop, the product of the roots is negative.* Thus, in the case of one real and two complex conjugates roots, the real root must be negative (the product of complex conjugates is always positive), whereas the real part of the complex conjugate roots may be either negative or positive. If it is negative, all three roots have negative real parts, and the steady state is *stable*. However, it is neither a pure node nor a pure focus. In the vicinity of the steady state, a perturbation will regress directly along one direction and periodically along a surface normal to this direction.

3. Limit Cycle

The case of complex roots with a positive real part is especially interesting. The steady state, called a saddle-focus, is attractive along one direction, but repulsive elsewhere. If one removes the system slightly from its steady state in a direction different from the attractive one, it will depart from the steady state, initially following a spiral expansion. On the other hand, as in all the bounded systems we consider, any point located outside a certain "box" will tend to reenter the box. This can be seen from Equation(s) (5). The term $k_1 F_1(z)$ has a maximal value k_1 , so whenever $x > k_1/k_{-1}$, the derivative dx/dt will be negative and x will decrease. This same reasoning holds for the other variables. The boundary "box" in question is thus the rectangular parallelepiped whose diagonal goes from $(0, 0, 0)$ to $(k_1/k_{-1}, k_2/k_{-2}, k_3/k_{-3})$. If all three equations were linear, the system would be unbounded and there would not be such a constraint, but it suffices that *one* equation be sigmoid to impose it.

The remarkable result is that, instead of pursuing its spiral expansion to infinity, the system asymptotically approaches a closed curve (Figure 6b) called the *limit cycle* (Poincaré). Furthermore, the same closed curve would also be approached from initial states located outside the cycle (Figure 6a). Thus, an unstable steady state in a bounded system can give rise to stable periodic behavior.

In principle, a point located exactly on the privileged attractive line would proceed to the steady state and remain there, but such mathematical precision is not encountered in real situations. A point close to the attractive line would first proceed toward the steady state, then depart from it, and periodically approach the limit cycle (Figure 6c). The evolution of the same system is seen in Figure 7a and b.

The *analytic* demonstration that a system can have a limit cycle has been carried out only in a small number of cases.⁵ So far, there is no general demonstration for simple negative loops with three or more elements. However, numerical methods show that simple bounded negative loops have a limit cycle whenever their steady state is unstable.

Such final states of a system are called *attractors*. A stable steady state is a point attractor. A limit cycle is a cyclic attractor; in this case the attractor is organized around a steady state, but the steady state, being unstable, is not itself the attractor. Note that in the case just discussed, there is a single attractor — the limit cycle — organized around the unique unstable steady state, and the system approaches its limit cycle whatever the initial state, whether close to the steady state or far "outside" the region of the limit cycle (Figure 6a and 7a).

The important point here is that *for proper values of the parameters, a three- (or, for that matter, more) element negative loop will respond to a constant environment with permanent oscillatory behavior.*

What is meant by "proper values of the parameters"? First of all, there are conditions (discussed in Section 4) which must be fulfilled for the negative loop to be effective, i.e., to ensure homeostasis. In addition, there is a condition of *minimal nonlinearity*. If one uses

* The product P of the roots equals $a_{11}a_{22}a_{33} - a_{12}a_{23}a_{31} + a_{13}a_{21}a_{32}$. The first term is the product $(-k-1) \cdot (-k-2) \cdot (-k-3)$ and is thus negative. The second term is zero. The third comprises an odd number of negative factors (only $F(z)$ has a negative derivative) and is thus also negative. Therefore, P is negative.

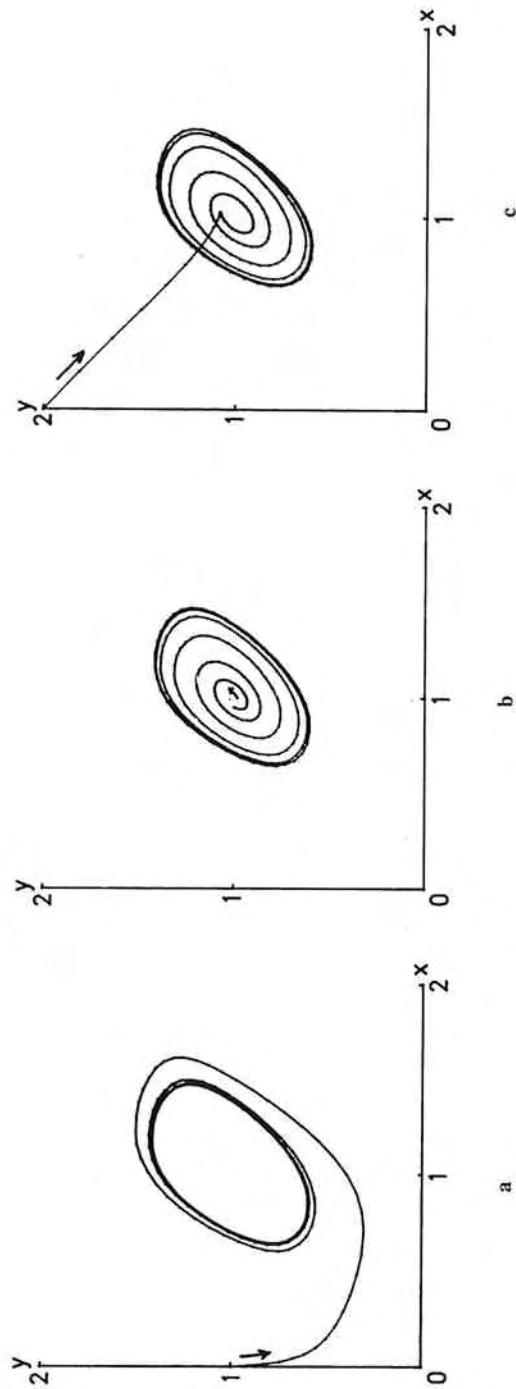


FIGURE 6. A limit cycle. Here, we have a three-dimensional system. The trajectories are projected on plane x - y . (a, b) Either "from outside" or "from inside", the system tends to the limit cycle. (c) Here, the initial state is very close to the attractive line. The system first proceeds toward the steady state, then periodically departs from it and approaches the limit cycle. Parameter values are $k'_s = 2$, $k_s = 1$, $\theta_s = 1$, $n = 5$. The initial states are (a) $(0, 2, 0)$, (b) $(0.95, 1.0, 1.05)$, and (c) $(0, 2, 0)$.

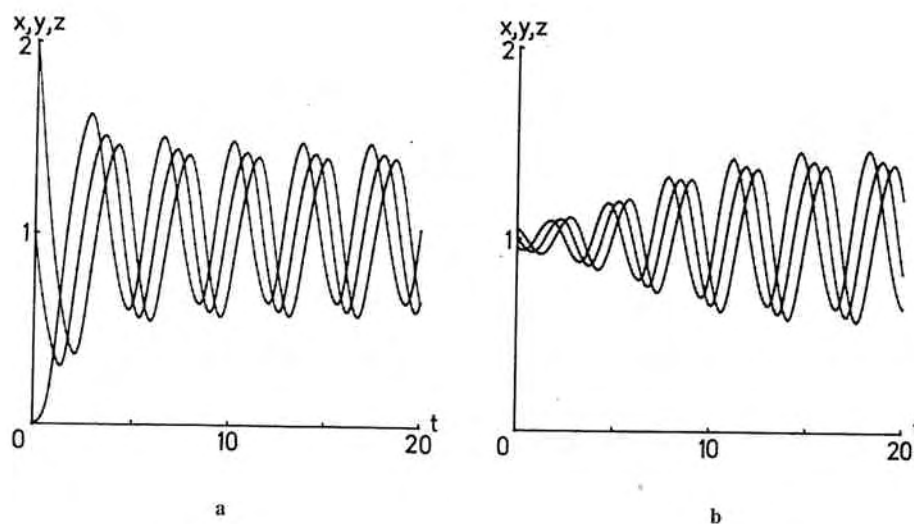


FIGURE 7. Evolution of the system whose trajectories are given in Figure 6a and b. Initial states: (a) (0, 1, 2), (b) (0.95, 1.0, 1.05).

Hill sigmoids for all three interactions, there is a minimal value of n below which the steady state is stable and, consequently, without permanent oscillations along a limit cycle. As shown by Richelle⁴ for a three-element system, the *product* of the n 's must be greater than 8 to obtain oscillatory behavior. Thus, if equal values are used for the n 's in a three-element system, one needs $n > 2$ (a necessary, but not sufficient, condition).

Earlier studies¹⁻³ considered three-element loops with two positive linear interactions and one negative nonlinear interaction. This is because they wanted to represent the concrete situation of a substance \underline{x} which is transformed into \underline{y} (thus, $dy/dt = k_2x - (k_3 + k_{-2})y$) which, in turn, is transformed into \underline{z} ($dz/dt = k_3y - k_{-3}z$); \underline{z} is a negative regulator of the synthesis of \underline{x} ($dx/dt = k_1F(z) - (k_2 + k_{-1})x$). In this case, the only nonlinear term must be highly nonlinear in order to generate stable oscillations. For a three-variable system, there is no limit cycle unless $n > 8$. But, as seen above, the nonlinearity can be spread among the interactions of the loop such that the fatidic "8" is surpassed by the product of three weak ($n = 3$) nonlinearities rather than by a single strong ($n > 8$) nonlinearity.

Finally, it must be stressed that in real systems there are often absolute time delays, so that instead of writing

$$x(t) = f(x, y, z, \dots)_t$$

one should write

$$x(t) = f(x_{t-t_1}, y_{t-t_2}, z_{t-t_3}, \dots).$$

For such systems, oscillations can occur in a wider domain of three-element loops and, in fact, there can be stable oscillations with two- and even one-element loops.⁴

4. Under What Conditions Will Homeostasis Be Effective?

As with one- and two-element loops, it is useful to consider the limit case of steep (step function-like) sigmoids to ask under what conditions homeostasis is effective. The condi-

tions are readily seen to be

$$\frac{k_1}{k_{-1}} > \theta_x, \quad \frac{k_2}{k_{-2}} > \theta_y, \quad \frac{k_3}{k_{-3}} > \theta_z.$$

As before, this means that the boundary level $\frac{k}{k_-}$ of each variable must exceed its threshold θ . If one or more of these conditions are not fulfilled, the steady state will be a stable node whose coordinates, depending on the variable, will be near 0 or near $\frac{k}{k_-}$ (unregulated values, no homeostasis). If all three conditions are fulfilled, the steady state will be a focus whose coordinates will be close to $(\theta_x, \theta_y, \theta_z)$ (homeostasis effective). This focus may be stable, in which case the attractor is the steady state itself, or it may be unstable, with the attractor a limit cycle.

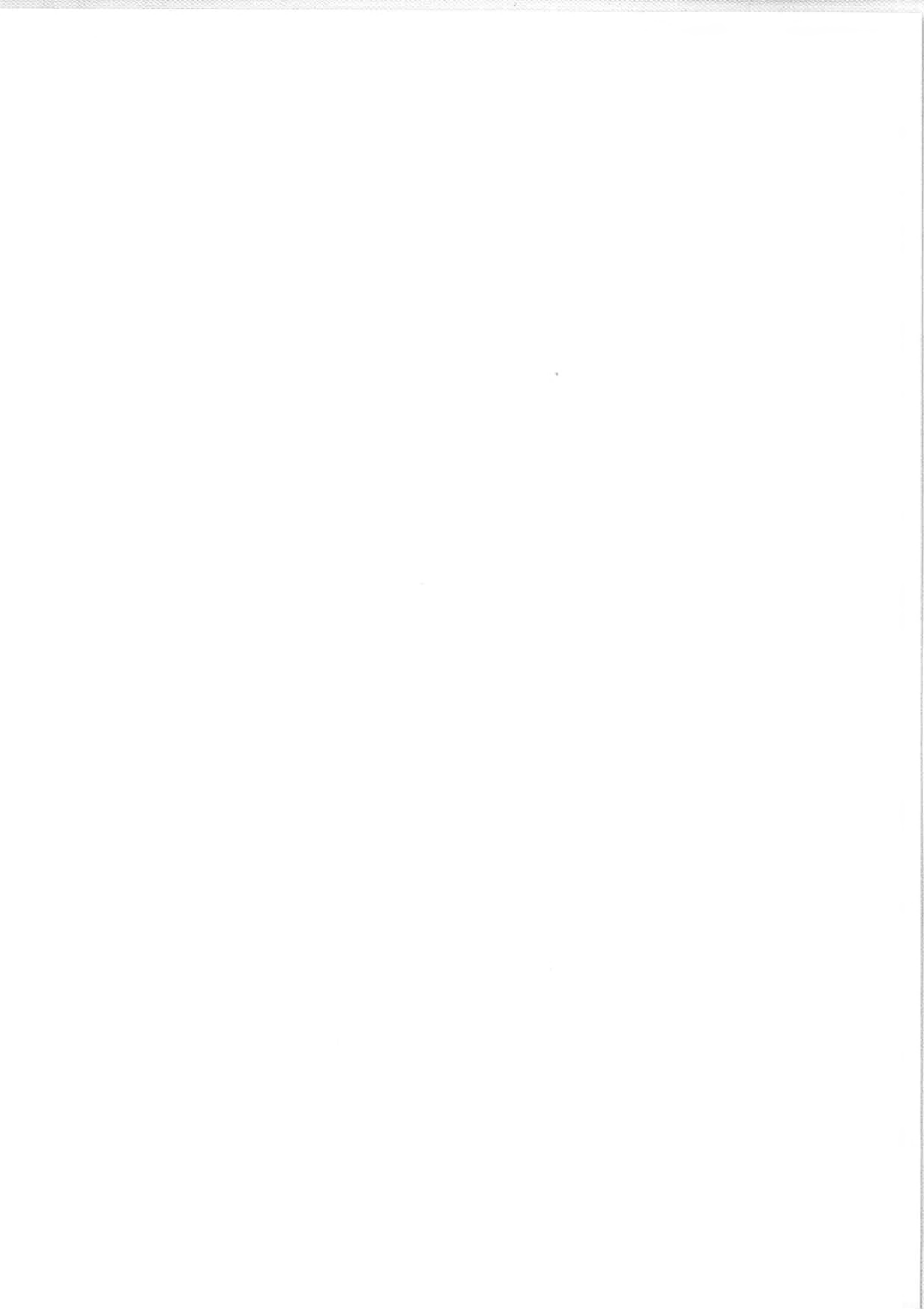
III. SUMMARY

The above illustrates the fact that a system consisting of an effective simple negative loop has a single steady state and that, depending on the parameters, it will approach either this steady state itself (if the steady state is stable) or a limit cycle (if the steady state is unstable). In either case, the system approaches or oscillates around a steady-state value. This is precisely the behavior of a thermostat, which ideally would stabilize *at* the desired temperature, but in practice oscillates around it — and a thermostat is, indeed, a simple negative loop.

We would characterize the operation of a simple negative loop by saying that it tends to keep the value of one or more variables at or in the vicinity of a supposedly optimal value: this is *homeostasis*. The amplitude of the oscillations (if any) depends on the values of the parameters. In the case of a thermostat, the smaller the oscillation, the better. This is not necessarily so in biological systems, for which, in many cases, the occurrence of the oscillations is a fundamental feature of the system, rather than an indication that the homeostatic control is too loose!

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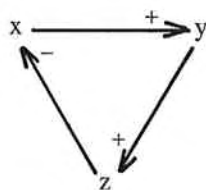
Chapter 11

**SIMPLE NEGATIVE FEEDBACK LOOPS GENERATE
HOMEOSTASIS: LOGICAL DESCRIPTION****TABLE OF CONTENTS**

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I. NAÏVE LOGICAL DESCRIPTION

We shall first consider the simple three-element negative loop:



and write that the production of element \underline{x} is on ($X = 1$) if element \underline{z} is absent ($z = 0$), etc., according to the *naïve logical description*:

$$X = \bar{z}$$

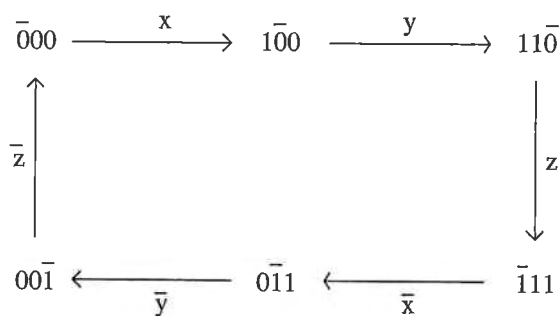
$$Y = x$$

$$Z = y$$

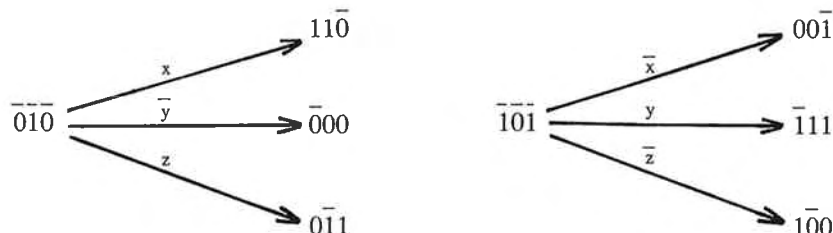
The state table is

xyz	XYZ
$\bar{0}00$	100
$00\bar{1}$	000
$0\bar{1}1$	001
$\bar{0}\bar{1}\bar{0}$	101
$11\bar{0}$	111
$\bar{1}11$	011
$\bar{1}\bar{0}\bar{1}$	010
$1\bar{0}0$	110

The graph of sequences of states comprises a cycle:



Once in the cycle, the system can only go on cycling since each state of the cycle has only a single order to change a variable and, thus, only one possible successor. From states $\bar{0}\bar{1}\bar{0}$ and $\bar{1}\bar{0}\bar{1}$, the system can proceed to any of three states in the cycle:

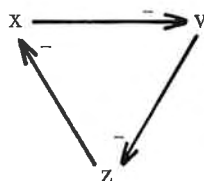


(States like $\bar{0}\bar{1}\bar{0}$ and $\bar{1}\bar{0}\bar{1}$ are sometimes called “Gardens of Eden” because one can leave them, but they cannot be reached from any other state.)

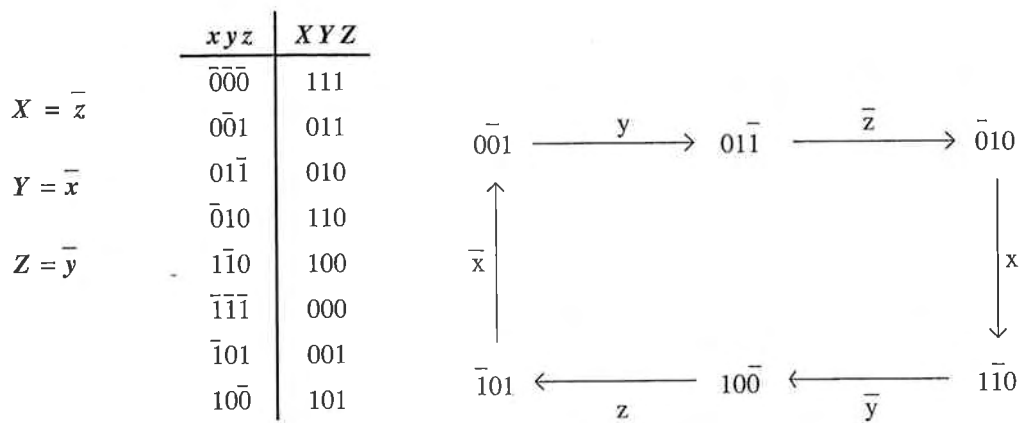
In terms of time delays, it is obvious that once a state in the cycle is reached, the system will follow the cycle *whatever the values of time delays*. From state $\bar{0}\bar{1}\bar{0}$, the system will proceed to $1\bar{1}\bar{0}$, $\bar{0}\bar{0}\bar{0}$, or $0\bar{1}\bar{1}$ according to the relative values of t_x , $t_{\bar{y}}$, and t_z , and from state $\bar{1}\bar{0}\bar{1}$ it will proceed to $0\bar{0}\bar{1}$, $\bar{1}\bar{1}\bar{1}$, or $\bar{1}\bar{0}\bar{0}$ according to the relative values of $t_{\bar{x}}$, t_y , and $t_{\bar{z}}$.

Recalling that $x = 0$ or $x = 1$ means that the concentration of the product \underline{x} is less than or greater than a threshold ϑ_x , the cycle around means that the concentrations of products \underline{x} , \underline{y} , and \underline{z} oscillate around their respective threshold values ϑ_x , ϑ_y , ϑ_z — typical homeostatic behavior.

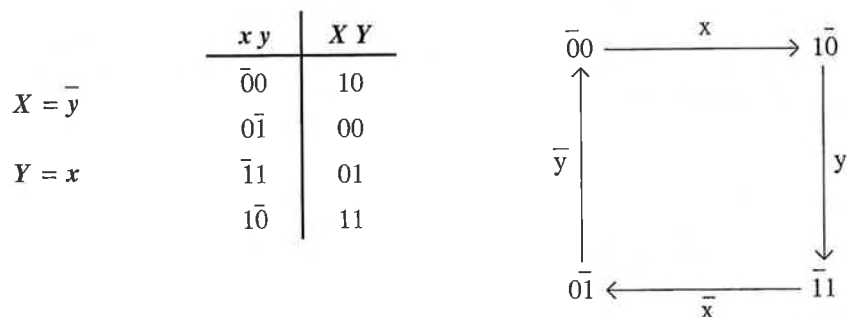
The simple three-element loop with three negative interactions is formally equivalent to the one with a single negative interaction (as pointed out in Chapter 9, Section II), from which it can be obtained by simply substituting $y = \bar{v}$:



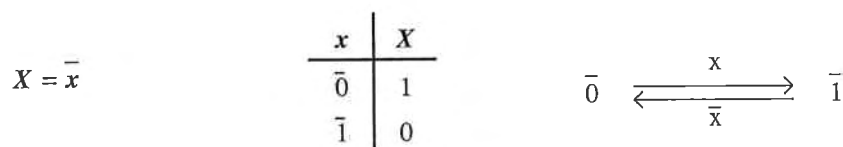
In practice, we prefer to maintain the convention that when a variable takes the value 0, the corresponding element is absent. However, this type of formal treatment, in which $v = 0$ means that \underline{y} is present, is useful in that it tells us that this loop, like the preceding one, will have an inescapable cycle of six states:



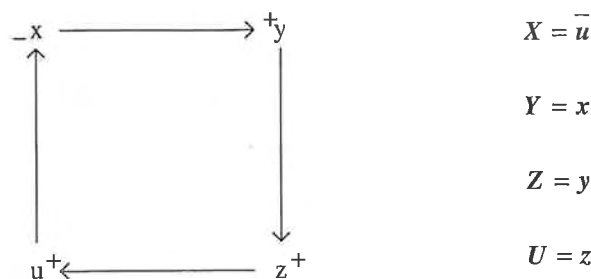
The behavior of the two-element negative loop, $x \xrightarrow{+} y \xrightarrow{-} x$, has already been discussed (Chapter 3, Section II):



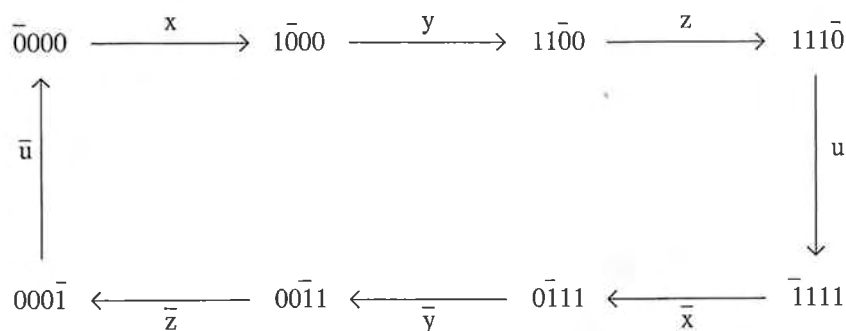
The one-element negative loop, $x \xrightarrow{-} x$ also oscillates:



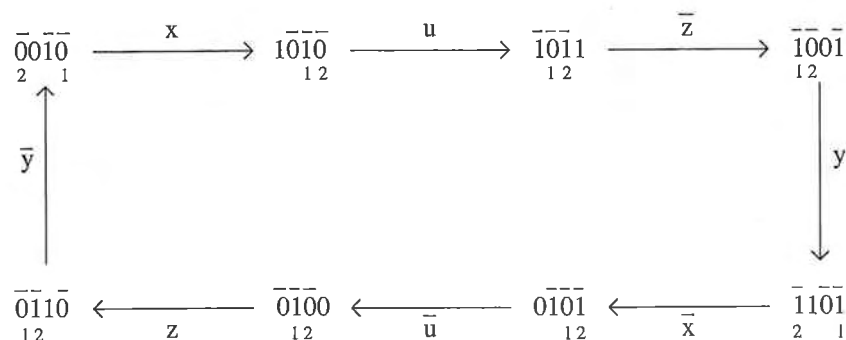
In m -element simple negative loops with more than three elements ($m > 3$), we again find a simple cyclic attractor with $2m$ states. For instance, the network:



has the cyclic attractor:



This cycle, like that of the three-element negative loop, cannot be left, once entered. In this system, we also find another cycle, which can be shown to be unstable. The sequence is



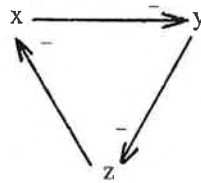
Note that from each state the system can either follow the unstable cycle or "fall" into one of two states of the attractor cycle. Each state has variables with subscripts — a characteristic of unstable cycles — and, in fact, the time delays form two overlapping circuits (cf. Chapter 4, Section III).

II. GENERALIZED LOGICAL DESCRIPTION

The reader will have noticed that the "naïve" logical description of simple negative loops discussed above unconditionally predicts cyclic behavior, whereas the continuous description (Chapter 10) predicts oscillating behavior only if certain conditions are fulfilled. In particular, a necessary (although not sufficient) condition for oscillating behavior is that the boundary k/k_- of each variable be sufficiently greater than the threshold value ϑ .

The difference is due to the fact that in the naïve logical description we have tacitly assumed that each term of the logical expression is strong enough to be taken into consideration (equivalent to assuming that each k/k_- is sufficiently greater than the corresponding ϑ). The situation can be generalized by introducing logical parameters K (according to Snoussi¹), which qualify the strength of each term, as explained in Chapter 7. This is useful even in the case of a simple negative loop, in which all variables are binary.

We will illustrate the generalized treatment with the simple three-element negative loop with three negative interactions:



The relations become:

$$X = d_x(K_1 \bar{z}) = K_1 \bar{z},$$

$$Y = d_y(K_2 \bar{x}) = K_2 \bar{x},$$

$$Z = d_z(K_3 \bar{y}) = K_3 \bar{y},$$

in which K_1 , K_2 , and K_3 each have the value 1 or 0, according to whether the term in question must effectively be taken into consideration. Comparing with the differential treatment, $K_1 = 1$ means that k_1/k_{-1} is sufficiently greater than ϑ_x , etc. The state table becomes:

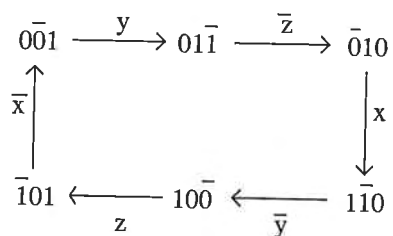
xyz	X	Y	Z
000	K_1	K_2	K_3
001	0	K_2	K_3
011	0	K_2	0
010	K_1	K_2	0
110	K_1	0	0
111	0	0	0
101	0	0	K_3
100	K_1	0	K_3

Since there are three binary parameters, this description covers 2^3 distinct situations, one of which ($K_1 = K_2 = K_3 = 1$) corresponds to the "naïve" description. Let us examine these situations. Because of the symmetry of the system, what is true of one element will be true of the others, *mutatis mutandis*, so there are only four different possibilities, according to whether 0, 1, 2, or 3 of the logical parameters are equal to 0.

$$K_1 = K_2 = K_3 = 1$$

$$\begin{aligned} X &= \bar{z} \\ Y &= \bar{x} \\ Z &= \bar{y} \end{aligned}$$

xyz	XYZ
$\bar{0}\bar{0}\bar{0}$	111
$\bar{0}\bar{0}1$	011
$\bar{0}1\bar{1}$	010
$\bar{0}10$	110
$1\bar{1}0$	100
$1\bar{1}\bar{1}$	000
$1\bar{0}1$	001
$10\bar{0}$	101



$$K_1 = 0$$

$$K_2 = K_3 = 1$$

$$X = 0$$

$$Y = \bar{x}$$

$$Z = \bar{y}$$

xyz	XYZ
$\bar{0}\bar{0}\bar{0}$	011
$\bar{0}\bar{0}1$	011
$\bar{0}1\bar{1}$	010
$\bar{0}10$	010
$1\bar{1}0$	000
$1\bar{1}\bar{1}$	000
$1\bar{0}1$	001
$10\bar{0}$	001

Similarly, if $K_2 = 0$ and $K_1 = K_3 = 1$, we find that $\bar{0}01$, and if $K_3 = 0$ and $K_1 = K_2 = 1$, we find 100 .

$$K_1 = K_2 = 0$$

$$K_3 = 1$$

$$X = 0$$

$$Y = 0$$

$$Z = y$$

xyz	XYZ
000	001
001	001
011	000
010	000
110	000
111	000
101	001
100	001

$$K_1 = K_2 = K_3 = 0$$

$$X = 0$$

$$Y = 0$$

$$Z = 0$$

xyz	XYZ
000	000
001	000
011	000
010	000
110	000
111	000
101	000
100	000

Similarly, if $K_2 = K_3 = 0$ and $K_1 = 1$, we find

that 100; if $K_1 = K_3 = 0$ and $K_2 = 1$, we

find that 010.

Thus, the generalized logical description shows that this loop will generate cyclic behavior only if all three logical parameters have the value 1, in other words, provided all three terms are strong enough. In the other cases, the system has a single stable state whose location depends on which interactions are effective. Since the simple three-element loop with three negative interactions is formally equivalent to the one with one negative and two positive interactions, our conclusion holds for the latter as well; cyclic behavior will only be observed if all three terms are sufficient (i.e., $K_1 = K_2 = K_3 = 1$).

More generally, the three-element negative loop can achieve the homeostasis (with a possible oscillation of the variables) only if all three terms are strong enough. In this case, we say the loop is *efficient*. Otherwise, homeostasis will not take place in the sense that each of the variables will be blocked in either the "on" or the "off" position.

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**POSITIVE FEEDBACK LOOPS GENERATE
DIFFERENTIATION: DIFFERENTIAL DESCRIPTION**

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I. INTRODUCTION

Until relatively recently, it was believed that any system would tend sooner or later toward a unique equilibrium state. It is now clear (for references, see Prologue) that open systems can display much more interesting behavior, such as permanent periodicity in a constant environment or a choice between two or more permanent steady states. The latter property (multistationarity) is of special interest to biologists, who were desperately trying to find out how genetically identical cell lines can display different heritable phenotypes in the same environment. This is the fundamental problem of differentiation.

We have already indicated on intuitive grounds, and shown by examples, that multistationarity can be generated by a simple positive feedback loop. This will be developed in a more rigorous way in this chapter (differential description) and in Chapter 13 (discrete description). In Chapter 14, we will show how one can have *many steady states*, as required to account for the many states of differentiation of higher organisms.

II. THE SIMPLE ONE-ELEMENT POSITIVE LOOP

The simple one-element positive loop describes *direct autocatalysis*. The most straightforward example is perhaps provided by proteolytic enzymes that are produced from a precursor and that catalyze their own formation by proteolytic action on their precursor:



(Here, the double arrow symbolizes the *conversion* of a into x , whereas the single arrow represents the regulatory interaction proper.) This type of process is called *product activation*. If a is maintained constant, the system can be treated as a simple, one-element, positive loop in which x behaves autocatalytically.

Another, related process is *substrate inhibition*:



If x inhibits its own conversion into b , it behaves autocatalytically in the sense that the more x present, the slower its conversion into b .

It has also become clear that certain gene products exert a positive effect on their own production. Strictly speaking, this situation is not a one-element loop because gene expression usually involves the syntheses of two successive products, the messenger RNA and a polypeptide chain. As a matter of fact, people interested in details would even argue that each of these syntheses itself comprises many steps. However, if one wants to analyze the global behavior of complex gene networks, it is indispensable to simplify some aspects. Insofar as a given gene is regulated predominantly at a single level (which may differ from gene to gene), we consider it legitimate in many cases to formalize each gene interaction as a single process. Thus, the case just discussed can be represented:



Although the mechanisms of Relations 1, 2, and 3 are different, they clearly have a common graph of interaction:



which is characteristic of the one-element positive loop.

In the differential description of the latter process, we write:

$$\frac{dx}{dt} = H(x) = kF^+(x) - k_-x \quad (4)$$

in which, as before, F^+ is an increasing Hill function, k and k_- are positive kinetic constants, and x , the concentration of product \underline{x} , is nonnegative. Relation 4 means that product \underline{x} is synthesized at a rate which is nil in the absence of \underline{x} and which approaches k for high concentrations of \underline{x} ; and \underline{x} decays at a rate proportional to its own concentration.

The steady-state equation can be written

$$H(x) = kF^+(x) - k_-x = 0$$

or

$$x = \frac{k}{k_-} F^+(x) = G^+(x).$$

In order to find the steady states, one can plot $H(x)$ as a function of x (Figure 1A) and find the intersects with the x axis, or plot $G^+(x) = \frac{k}{k_-} F^+(x)$ as a function of x and find the intersects of $G(x)$ with the bissectrix x (Figure 1B). As $G^+(x)$ is an increasing sigmoid, it is clear that it can have up to three intersects with the bissectrix x . *There can thus be up to three steady states.* This important property is called *multistationarity*.

For any given values of the parameters k/k_- , θ_x , and n , the steady-state values can be found numerically (see Appendix 1).

The stability properties of these steady states are illustrated by the case chosen in Figure 1. As shown in Chapter 6, for a one-variable system, a steady state x^0 is stable or unstable, depending simply on whether the slope of $H(x)$ at x^0 is negative or positive. In the example of Figure 1, the outer steady states are thus stable and the intermediate one unstable. Note that:

$$\frac{dH}{dx} = \frac{k n \theta^n x^{n-1}}{(\theta^n + x^n)^2} - k_-.$$

The first term is the derivative of a sigmoid, a bell-shaped curve with very low values for extreme values of x and high values only in the vicinity of θ . (More precisely, the maximum corresponds to the inflection point of H , which, for high n , is close to θ .) It is thus understandable that $\frac{dH}{dx}$ is negative for the outer steady states and positive for the intermediate one.

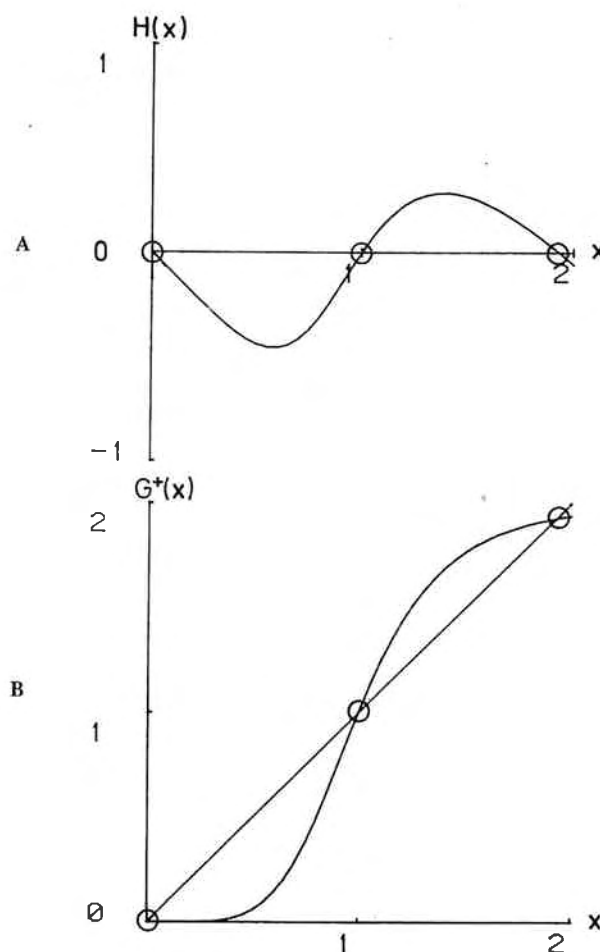


FIGURE 1. One-element positive loop. (A) Plot of $H(x)$ as a function of x . The steady states are the values of x at the intersects of $H(x)$ and the x axis. As shown in Chapter 6, the stability of each steady state (x^0) depends simply on the slope of $H(x)$ at x^0 : stable if the slope is negative, unstable if it is positive. (B) Plot of $G^+(x) = \frac{k}{k_-} F^+(x)$ as a function of x . Here, the steady states are given by the intersects of $G^+(x)$ with the bisectrix. In our particular case, $H(x) = \frac{2x^5}{1+x^5} - x$ and, thus, $G^+(x) = 2 - \frac{x^5}{1+x^5}$.

What determines how many states there are? As with negative loops (Chapter 10), the situation is simplest in the case of extremely steep sigmoids, in which case it is clear that there will be three steady states iff $k/k_- > \theta$ (Figure 2). For finite n , there will be three steady states iff $k/k_- > a_n \theta$, where a_n increases from 1 to 2 as n decreases from ∞ to 2.* Whereas for negative loops the parameter values affect the *nature* of the unique steady state, for positive loops they affect the *number* of steady states.

III. THE TWO-ELEMENT POSITIVE LOOP

As a second example, we consider the positive loop $x \rightleftarrows y$. This situation exists in nature. For example, the bacteriophage λ has two genes, *cI* and *cro*, whose products

* It can be shown that $a_n = \frac{n}{(n-1)(n-1)/n}$. (Kaufman, M., personal communication.)

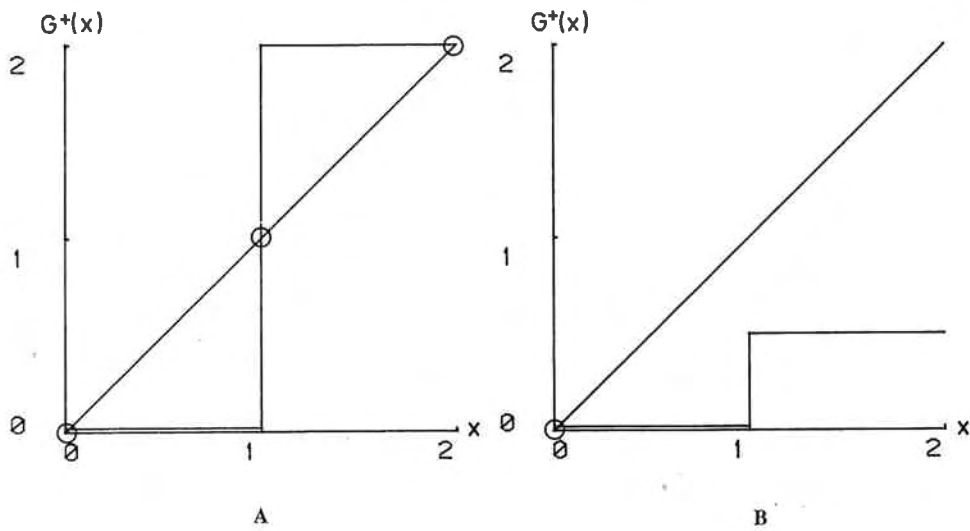


FIGURE 2. How many steady states? Plot of $\frac{k}{k_-} F^+(x)$ as a function of x , for extremely steep sigmoids ($n \rightarrow \infty$).

$$(A) G^+(x) = \frac{2x^n}{1 + x^n}$$

$$(B) G^+(x) = \frac{0.5x^n}{1 + x^n}$$

In the first case, $\frac{k}{k_-} > \theta$ and there are three intersects with the bissectrix. In the second case, $\frac{k}{k_-} < \theta$ and there is only one intersect.

each exert a negative control on the other gene, with the type of result described below. The equations are

$$\frac{dx}{dt} = k_1 F^-(y) - k_{-1} x = H_x(x, y)$$

$$\frac{dy}{dt} = k_2 F^-(x) - k_{-2} y = H_y(x, y)$$

with the same notation and meaning as above.

The steady-state equations, defined by

$$\frac{dx}{dt} = 0 \quad \text{and} \quad \frac{dy}{dt} = 0,$$

are

$$x = \frac{k_1}{k_{-1}} F_1^-(y) = G_1^-(y)$$

$$y = \frac{k_2}{k_{-2}} F_2^-(x) = G_2^-(x)$$

(5)

Thus,

$$x = G_1^{-1}[G_2(x)] = G_3(x), \quad (6)$$

and similarly for y .

As mentioned in Chapter 6, the composition of Hill functions (for instance $G_1^{-1}[G_2(x)]$), gives functions of a sigmoid shape, and when an *even* number of decreasing Hill functions is involved, the resultant function is an increasing sigmoid ($G_3^+(x)$). The roots of Equation 6 are the steady-state values of variable x . They can be visualized (see Figure 3) as the intersects of x (the bisectrix) with $G_3^+(x)$. For the parameter values chosen, there are three intersects; more generally, from the shape of $G^+(x)$, it is intuitively clear that the number of intersects can vary from one to three according to the parameter values. Thus, in this system, as with the one-element positive loop, we can have up to three distinct steady states.

In Figure 4, the steady state Equation(s) 5 are drawn in the x - y plane for the same parameter values as in Figure 3. The two curves (nullclines) correspond to the situations $dx/dt = 0$ and $dy/dt = 0$, and their intersections thus locate the steady states, where *both* conditions are fulfilled. In the present case, as can be seen, the steady states correspond to low x and high y (State 1), middle values of both x and y (State 2), and high x and low y (State 3). The exact values can be calculated numerically, as described in Appendix 1.

Taking our example with three steady states, linear stability analysis (see Appendix 3, Example 3) shows that two steady states (1 and 3) are stable and one (2) unstable. For State

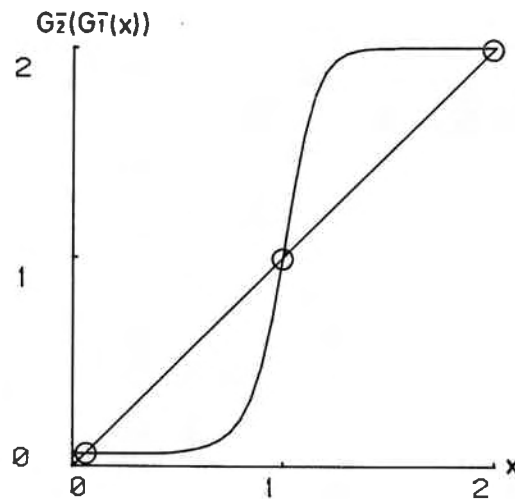


FIGURE 3. Two-element positive loop: plot of $G_3(x) = G_1(G_2(x))$. In our particular case,

$$\frac{dx}{dt} = \frac{2}{1+y^5} - x$$

$$\frac{dy}{dt} = \frac{2}{1+x^5} - y$$

The steady state equations are thus:

$$x = \frac{2}{1+y^5} = G_1(y)$$

$$y = \frac{2}{1+x^5} = G_2(x)$$

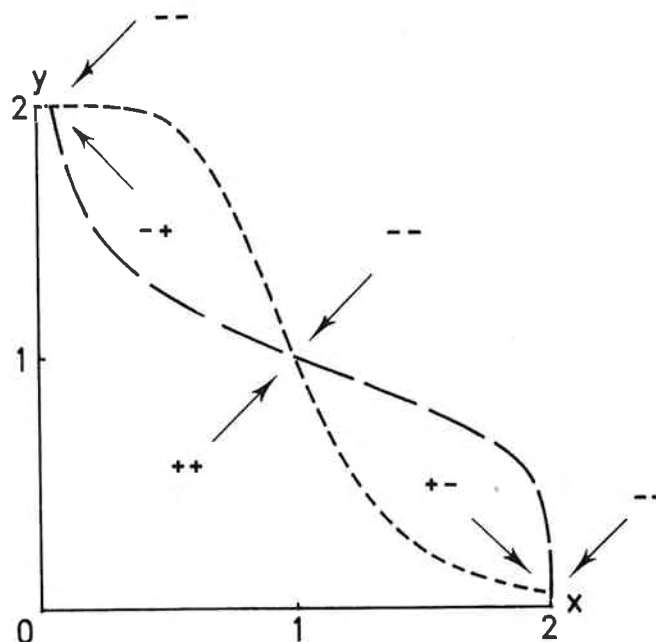


FIGURE 4. Two-element positive loop. The nullclines of the system described in Figure 3.

$$---, \frac{dx}{dt} = 0$$

$$—, \frac{dy}{dt} = 0$$

2, the roots are real and of opposite sign; it is a *saddle point*. The physical meaning of this situation will become more apparent if we compare the x - y plane with the map of a mountain landscape comprising two closed valleys (whose lowest points correspond to the stable nodes) separated by a ridge whose lowest point, or pass, corresponds to the saddle point. Two lines, called *separatrices*, have a special importance. They cross each other at the saddle point. One of them, which we call “main” separatrix, or simply separatrix, coincides with the ridge; the other joins the two stable states via the saddle point. A ball freely rolling on this landscape would finally reach one of the stable states according to whether it was on one or the other side of the ridge. The two stable states are *attractors*, and the two basins of attraction are delimited by the ridge.

If our system were exactly *at* the saddle point, it would remain there. Moreover, if it were exactly *on* the ridge, it would approach the unstable state along the main separatrix and remain there. However, the slightest deviation to one side or the other would commit the ball to proceed toward the corresponding stable state (valley). The saddle point is thus not an attractor; the two stable states are the two *attractors* of the system (Figure 5).

The conditions for having three steady states, i.e., the condition for the loop to effectively generate multistationarity, is that each boundary value k/k_0 be sufficiently greater than the corresponding threshold θ .

The concrete example mentioned at the beginning of this section was that of the λ phage genes *cI* and *cro*, each of which is repressed by the product of the other. In agreement with the above, the system can reach and persist in either of the two stable (hereditary) states, one with *cI* expressed and *cro* silent, the other with *cI* silent and *cro* expressed. The major inter-

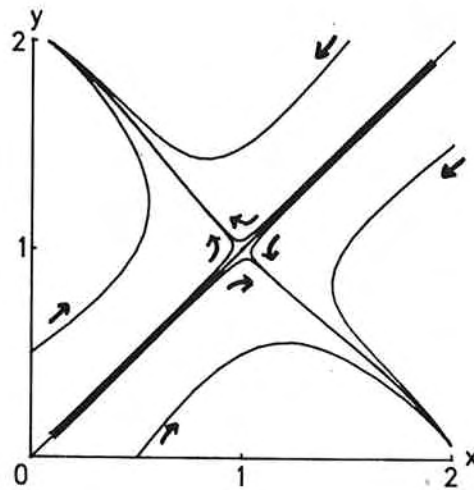
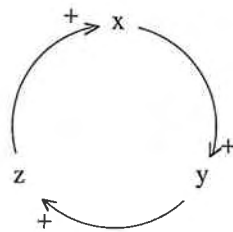


FIGURE 5. Trajectories of the system described in Figures 3 and 4.

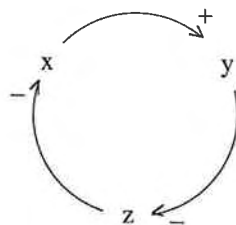
est of such a structure is that, despite its simplicity, it endows the system with the possibility to respond to identical external conditions with two widely different steady states.

IV. MULTIPLE-ELEMENT POSITIVE LOOPS

Let us rapidly treat the symmetric three-element positive loop:



The asymmetric loop



can be derived from it by a simple change of variable (cf. Chapter 9). The differential equations are

$$\frac{dx}{dt} = k_1 F^+(z) - k_{-1}x$$

$$\frac{dy}{dt} = k_2 F_2^+(x) - k_{-2}y$$

$$\frac{dz}{dt} = k_3 F_3^+(y) - k_{-3}z$$

and the steady-state equations are

$$x = \frac{k_1}{k_{-1}} F_1^+(z) = G_1^+(z)$$

$$y = \frac{k_2}{k_{-2}} F_2^+(x) = G_2^+(x)$$

$$z = \frac{k_3}{k_{-3}} F_3^+(y) = G_3^+(y)$$

Again, we can write that $x = G_1^+(G_3^+(G_2^+(x))) = G_x^+(x)$ and similarly for y and z . As $G_x^+(x)$ is an increasing sigmoid function of x , there are up to, but not more than, three intersects between $y = x$ and $y = G_x^+(x)$. The three-element positive loop thus has up to three steady states, but not more, like the one- and two-element positive loops.

This result can be generalized to any simple positive loop (using sigmoid interactions), whatever the number of elements in the loop and whatever the steepness of the sigmoids. This point is important. One might have thought that the number of steady states could be increased simply by properly increasing the algebraic degree of the steady-state equations. In fact, for sigmoid interactions, any value of the Hill exponent $n > 1$ makes three steady states possible, but a further increase of n does not increase the number of steady states.

For a one-variable loop, the system will go to one or the other stable state according to whether it was initially on one or the other side of the unstable state. For a two-variable loop, the system will go to one or the other stable state according to whether it was initially on one or the other side of a separatrix line (on which the saddle point is located). For a three-variable system, it will go to one or the other stable state according to whether it is on one or the other side of a separatrix surface. More generally, for an n -variable system, it will go to one or the other stable state according to whether it is on one or the other side of a separatrix "hypersurface" ($n - 1$ dimensions for n variables) containing the unstable state. This view can be illustrated by linear stability analysis of a three-element positive loop. Let us consider the system:

$$\frac{dx}{dt} = \frac{2z^5}{1 + z^5} - x$$

$$\frac{dy}{dt} = \frac{3x^5}{1 + x^5} - y$$

$$\frac{dz}{dt} = \frac{4y^5}{1 + y^5} - z$$

The steady-state equations are

$$x = \frac{2z^5}{1 + z^5}$$

$$y = \frac{3x^5}{1 + x^5}$$

$$z = \frac{4y^5}{1 + y^5}$$

and the steady state values, found by iteration, are

	$x = 0.0$	$x = 0.813$	$x = 1.998$
(I)	$y = 0.0$	(II) $y = 0.786$	(III) $y = 2.908$
	$z = 0.0$	$z = 0.927$	$z = 3.980$

From these steady-state values and the Jacobian matrix* (cf. Appendix 3), we obtain the characteristic equation for each steady state:

(I)	$(\omega + 1)^3 = 0$	$\omega = -1$ (triple root)
(II)	$(\omega + 1)^3 = 41.94$	$\omega = 2.46$ and $-2.73 \pm 3.0i$
(III)	$(\omega + 1)^3 = 0.0000183$	$\omega = -0.97$ and $-1.01 \pm 0.022i$

This linear stability analysis confirms that States I and III are stable (all roots, or their real parts, are negative). For State II, we have a positive real root and two complex conjugate roots whose real part is negative. This steady state is therefore unstable. It is located on the surface separating the two attractors, I and III, and is at the same time a saddle point and a focus. It is attractive (in a periodic way) along the separatrix surface, but repulsive elsewhere. Thus if one starts from a point very close to the separatrix surface, the system will first proceed periodically toward the unstable steady state, then directly toward one of the stable steady states according to whether the initial state was on one or the other side of the separatrix.

* Jacobian matrix

$$\begin{bmatrix} -1 & 0 & \frac{10z^4}{(1+z^5)^2} \\ \frac{15x^4}{(1+x^5)^2} & -1 & 0 \\ 0 & \frac{\partial y^4}{(1+y^5)^2} & -1 \end{bmatrix}$$

Characteristic equation

$$\begin{vmatrix} -1 - \omega & 0 & \frac{10z^4}{(1+z^5)^2} \\ \frac{15x^4}{(1+x^5)^2} & -1 - \omega & 0 \\ 0 & \frac{\partial y^4}{(1+y^5)^2} & -1 - \omega \end{vmatrix} = 0$$

**POSITIVE FEEDBACK LOOPS GENERATE
DIFFERENTIATION: LOGICAL DESCRIPTION****TABLE OF CONTENTS**

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I. THE ONE-ELEMENT POSITIVE LOOP

The simple one-element positive feedback loop has the graph of interactions $x \xrightarrow{+} x$. It can represent, for example, a product \underline{x} whose synthesis requires the presence of \underline{x} . It is formalized:

$$X = d_x(Kx) = Kx$$

in which K is a logical parameter that takes the value 0 or 1 according to the weight ascribed to the term (cf. Chapter 7). The state tables are

General

x	X
①	0
1	K

Specific

$K = 0$

x	X
①	0
1	0

$K = 1$

x	X
①	0
①	1

Thus, provided $K = 1$, the one-element positive loop gives a choice between two stable states, ① and ①. As discussed in Chapter 8, Section V, the third, unstable state of the differential description can also be identified on logical grounds. If $K = 0$, there is only one stable state, ①. This corresponds to a situation in which the boundary concentration is insufficient to stimulate continued synthesis of \underline{x} . In the continuous description, this occurs when $\frac{k}{k_-} < \theta$, where k and k_- are kinetic constants for synthesis and decay of \underline{x} and θ is the threshold concentration above which the regulation is effective.

II. TWO-ELEMENT POSITIVE LOOPS

The two-element loop $x \xrightarrow{+} y \xrightarrow{+} x$, already treated in "naïve" terms (Chapter 3), can now be formalized:

$$X = K_{12}\bar{y}$$

$$Y = K_{21}\bar{x}$$

The general state table is

$x y$	X	Y
00	K_{12}	K_{21}
01	0	K_{21}
11	0	0
10	K_{12}	0

and the specific tables are

$$K_{12} = 1$$

$$K_{12} = 0$$

$$K_{12} = 1$$

$$K_{12} = 0$$

$$K_{21} = 1$$

$$K_{21} = 1$$

$$K_{21} = 0$$

$$K_{21} = 0$$

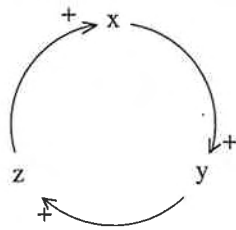
$x y$	$X Y$	$x y$	$X Y$	$x y$	$X Y$	$x y$	$X Y$
$\bar{0}\bar{0}$	11	$\bar{0}\bar{0}$	01	$\bar{0}\bar{0}$	10	$\textcircled{00}$	00
$\textcircled{01}$	01	$\textcircled{01}$	01	$0\bar{1}$	00	$0\bar{1}$	00
$\bar{1}\bar{1}$	00	$\bar{1}\bar{1}$	00	$\bar{1}\bar{1}$	00	$\bar{1}\bar{1}$	00
$\textcircled{10}$	10	$\bar{1}0$	00	$\textcircled{10}$	10	$\bar{1}0$	00

Thus, provided both terms have to be taken into consideration, (i.e., $K_{12} = K_{21} = 1$), we have two stable states, one with only \bar{x} present and continuously synthesized, the other with only \bar{y} present and continuously synthesized. This is also the result predicted by the naïve logical analysis (Chapter 3). Again, the third, unstable steady state of the differential description can also be identified on logical grounds (Chapter 8, Section V). If either of the terms is insufficient (K_{12} or $K_{21} = 0$), there is only a single stable state. At this point, we would like to insist on the importance of consulting the general state table, in which no definite numerical value is given to the logical parameters. It shows, for example, that in the fourth case ($K_{12} = K_{21} = 0$), the stable state $\textcircled{00}/00$ is more precisely $00/K_{12}K_{21}$ (with K_{12} and $K_{21} = 0$). Consequently, in the differential description, the steady state will be near $(k_{21}/k_{-1}, k_{21}/k_{-2})$, with $x^0 < \theta_x$ and $y^0 < \theta_y$, rather than near $(0, 0)$.

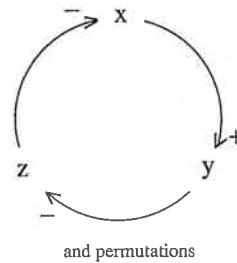
The two-element positive loop $x \begin{array}{c} \xrightarrow{+} y \\ \xleftarrow{+} x \end{array}$ presents the same type of behavior, as expected from the formal equivalence of the two types of two-element positive loops (cf. Chapter 9). There are two stable states, $\textcircled{00}$ and $\textcircled{11}$, if both logical parameters are 1, a single stable state otherwise.

III. THREE-ELEMENT POSITIVE LOOPS

The three-element positive loops are



and



Let us treat the second one:

$$X = K_{13} \bar{z}$$

$$Y = K_{21} x$$

$$Z = K_{32} \bar{y}$$

The general state table is

xyz	X	Y	Z
000	K_{13}	0	K_{32}
001	0	0	K_{32}
011	0	0	0
010	K_{13}	0	0
110	K_{13}	K_{21}	0
111	0	K_{21}	0
101	0	K_{21}	K_{32}
100	K_{13}	K_{21}	K_{32}

Once again, there are two stable states only for $K_{13} = K_{21} = K_{32} = 1$:

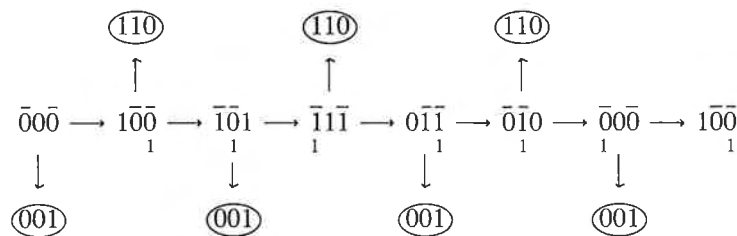
xyz	XYZ
$\bar{0}\bar{0}\bar{0}$	101
$\textcircled{001}$	001
$0\bar{1}\bar{1}$	000
$\bar{0}\bar{1}0$	100
$\textcircled{110}$	110
$\bar{1}\bar{1}\bar{1}$	010
$\bar{1}0\bar{1}$	011
$\bar{1}\bar{0}\bar{0}$	111

For other values of the parameters, there is only a single stable state:

K_{13}	K_{21}	K_{32}	Stable state
0	0	0	(000)
0	0	1	(001)
0	1	1	(001)
0	1	0	(000)
1	1	0	(110)
1	0	1	(001)
1	0	0	(100)

In the same way as in the preceding section, we would like to insist on the fact that if, for example, $K_{21} = 1$ and $K_{13} = K_{32} = 0$, the stable state (000) is more precisely $K_{13}00$ (with $K_{13} = 0$), which means that in the differential description this steady state will be near $(k_{13}/k_{-1}, 0, 0)$ (with $k_{13}/k_{-1} < \theta_x$) rather than near $(0, 0, 0)$.

For $K_{13} = K_{21} = K_{32} = 1$, the graph of sequences of states (starting from 000) is



In this graph, $\bar{0}\bar{0}\bar{0} \rightarrow \rightarrow \rightarrow \bar{0}\bar{0}\bar{0}$ is not a cycle, but $\bar{1}\bar{0}\bar{0} \rightarrow \rightarrow \rightarrow \bar{1}\bar{0}\bar{0}$ is treated as a cycle because its subscripts are equal (cf. Chapter 4, Section IV). It is, of course, an unstable cycle, not an attractor. Clearly, this unstable cycle is the Boolean equivalent of the saddle focus described in Chapter 12.

Another representation consists of embedding the graph on a cube (see References 1 and 2). The unstable cycle meanders around the "equator" of the cube, with the potential danger, in every state, of "falling" into one or the other "pole" (stable state). Although this representation is very vivid and has certain advantages, we usually prefer the type of graph given above because it permits one to distinguish two states that have the same Boolean value but different indices.

The other type of three-element positive loop, with three positive interactions, is formally equivalent to the one with two negative interactions. It has two stable states, (000) and (111), if all three logical parameters are 1, a single stable state otherwise.

The above example should convince the reader that the generalized description of a simple positive feedback loop will exhibit two stable states only if all terms are large enough that one has to take them into consideration (i.e., all logical parameters are 1). This is the situation in the naïve description and it corresponds to the condition that all k/k_- are sufficiently greater than the corresponding θ in the differential description. If one or more of these conditions are not fulfilled, there is a single stable state.

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2. Thomas, R. and Van Ham, P., Analyse formelle de circuits de régulation génétique: le contrôle de l'immunité chez les bactériophages lambdoides, *Biochimie*, 56, 1529, 1974.

Chapter 14

**HOW CAN A TRANSIENT SIGNAL SWITCH A FUNCTION
ON PERMANENTLY?****TABLE OF CONTENTS**

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I. THE PUSH-BUTTON PROBLEM

One of the most challenging aspects of cell differentiation is the fact that two cell lines, which, as far as one can tell, are genetically identical, heritably display different phenotypes. At first, one might be tempted to ascribe this to persistent environmental differences. That this usually is not the case is shown by the fact that the phenotypic differences may persist when cell lines are propagated *in vitro* under identical conditions. It is as though sometime during development a decision had been made and was subsequently maintained through cell generations. Embryologists use the word "determination" to describe this decision, which may take place long before the characteristic aspects of a cell line become visible ("differentiation").

Although little is known regarding the mechanisms of determination and differentiation, it is clear in many cases that a specific signal must function at some definite moment during development to permit a given type of differentiation event and that after this moment its function is no longer required and it is, in fact, switched off.

Similarly, in the induction of cancer by oncogenic viruses, cases are known in which a viral gene "transactivates" host genes involved in tumor development, then is itself switched off, whereas the host genes remain on.

We are thus led to ask: **how can a transient signal switch a gene on permanently and even hereditarily?** In other words, what logical mechanisms underlie a push-button device?

Consider a gene X that directly or indirectly exerts a positive control on its own expression. In the simplest version, the gene product is required for its own synthesis. This permits two stable states (on, off) and, as already mentioned, the situation is a typical vicious circle. Suppose, however, that gene X functions if its product x is present OR if another product a is present. This solves the vicious circle. Starting from a situation in which the gene is off and its product x and a are both absent, the gene will remain off indefinitely. If a is added, the gene will be switched on, its product x will appear, and from then on, the gene will remain on even if a disappears. Substance a functions as a trigger that is only necessary for switching the process on, not for its maintenance, which is subsequently ensured by the autocatalytic character of x synthesis.

This type of process is described by the simple logical relation:

$$X = x + a,$$

which says that gene X is on if substance a is present OR if its own product is present. In

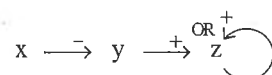
diagram form, $a \xrightarrow{+} x \xrightarrow{+} x$ (OR \rightarrow). Naturally, the condition that is used to trigger the process need not be simply " a is present"; it can require the simultaneous presence of several substances, the temporary absence of products normally present, or special environmental conditions (temperature, for example, is important in triggering the process of budding). Any or all of these processes can be the triggering condition. In all cases, the logical structure is the same (Table 1).

Clearly, starting from a stable state ① (gene off, its product absent, a absent), if one adds a (shift from left to right column), the state ② is reached and the gene product x will appear after a delay t_x , displacing us to line 2. From now on, the gene is on and will remain so even if substance a is removed since a shift back to the left column now leaves the system in stable state ①.

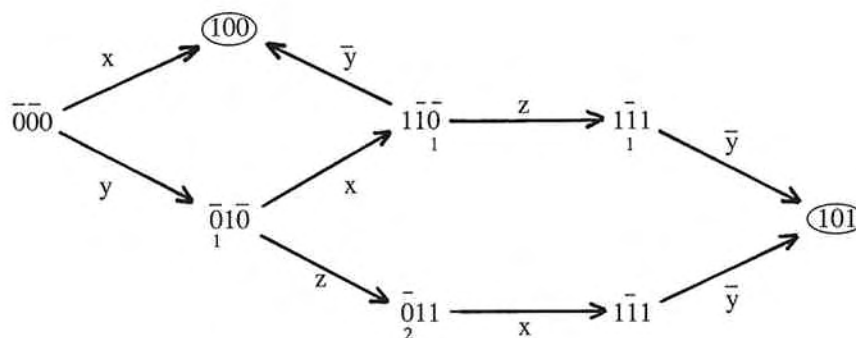
TABLE 1

$a = 0$		$a = 1$		Compact table
x	X	x	X	$0 \quad 1 \quad a$
0	0	0	1	0 \rightarrow 0
1	1	1	1	1 \leftarrow 1

Some situations of this type are known in prokaryotes. Temperate bacteriophage, for example, can elicit two different responses after infection of a sensitive bacterium. They can enter the lytic cycle, killing the host and producing more phage, or they can establish immunity, whereby a phage repressor gene is expressed, preventing the lytic cycle. Let us consider a simplified situation inspired from bacteriophage λ .



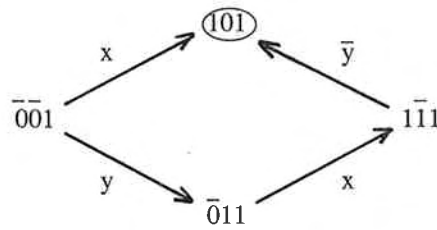
In other words, gene X is expressed constitutively, gene Y repressed by product x , and the expression of gene Z requires product y or its own product, z , which is, in fact, the phage repressor. This system was treated as an example in Chapter 4, Section II. Let us start from state $\bar{0}\bar{0}\bar{0}$, in which no phage gene product is present yet, resembling the situation just after infection. The graph of the sequences of states is



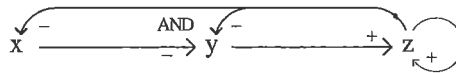
The interest of this simple system is the following: as gene x is constitutive, $X = 1$ from the beginning, and sooner or later x will appear. Thus, in the final state, one necessarily has $x = 1$. Gene Y will also be turned on initially ($Y = 1$) since x is absent. As soon as x appears, gene Y will be turned off ($Y = 0$), and sooner or later y , if present, will disappear. Thus, in the final state, one necessarily has $y = 0$. The situation is different for gene Z , the repressor gene. If, before x appears, y is actually produced and persists long enough not only to switch on gene Z but also to permit the accumulation of product z , gene Z will remain on indefinitely. If y disappears before z appears, gene Z will remain off indefinitely. Thus, depending on the precise kinetic history of the system, gene Z , coding for the phage repressor, will be on or off permanently, corresponding to the lysogenic or lytic response, respectively.

If we consider the initial state $\bar{0}\bar{0}\bar{1}$, in which product z (repressor) is already present in the bacterium at the time of infection, it can be seen that gene Z will remain on indefinitely.

There is no path leading from $\bar{0}\bar{0}1$ to the stable state (100) (lytic response); both choices end up at state (101) (lysogenic response).



We will now refine the system (and bring it closer to its concrete model) by adding that product z is a repressor that can switch off the regulatory genes X and Y :



Gene X is no longer constitutive, but is under the negative control of z , and gene Y is under negative control of both x and z . More precisely, Y is on iff x and z are absent. The (naïve) logical equations are

$$X = \bar{z}$$

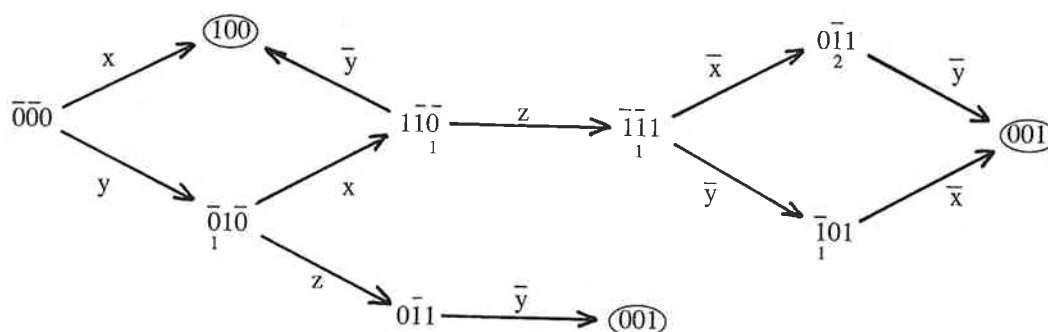
$$Y = \bar{x} \bar{z}$$

$$Z = y + z$$

and the state table is

xyz	XYZ
$\bar{0}\bar{0}0$	110
(001)	001
$0\bar{1}1$	001
$\bar{0}1\bar{0}$	111
$1\bar{1}\bar{0}$	101
$\bar{1}\bar{1}1$	001
$\bar{1}01$	001
(100)	100

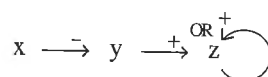
Again, there are two stable states, but they now resemble the actual choice by the bacteriophage: in state (001) , only the repressor gene is expressed and immunity is established, whereas in state (100) , the repressor is not synthesized, leaving the way open to the lytic cycle. The graph of the sequences of states is



As above, gene Z is switched on by product y ; but now, as soon as product z appears, gene Y (whose product is no longer required) is switched off.

This system is a fair caricature of the interactions between the genes *cro*, *cII*, and *cI* in bacteriophage λ (represented here by X , Y , and Z , respectively), which will be treated in more detail in Chapter 20.

The conditions leading to the different final states have been analyzed more formally (cf. Chapter 4, Section II) in the case of the original model. In fact, it can be shown¹ that the conditions determining whether immunity is established are exactly the same in this very simple model and in the more sophisticated one. This suggests that although the observed situation in bacteriophage λ is more complex, the essential principles of the *decision* whether to establish immunity are already present in the simple scheme:



II. VICIOUS CIRCULAR DNA?

In this connection, we have designed and, in collaboration with P. Drèze, D. Thieffry, N. Becker, and P. Campano, are trying to construct a small gene network that can be set on command in either of two stable positions by transient signals.

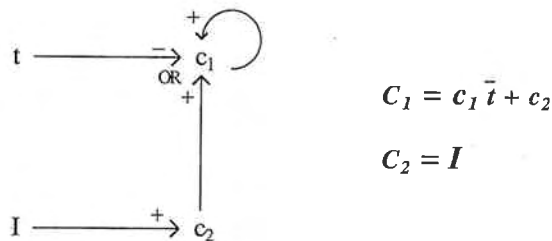
The λ gene *cII* is a positive control gene whose product is absolutely required for the operation of three promoters, called *pre*, *pI*, and *paQ*. If by *in vitro* recombination we remove the normal promoter of gene *cII* and replace it with a promoter (like *pI*) that requires the *cII* product, we have created a vicious circle: either *cII* product is present, in which case it will go on being synthesized, or it is absent, and since *cII* is necessary for transcription from promoter *pI*, it will continue not being synthesized and remain absent.

Consider, now, another copy of gene *cII*, again separated from its normal promoter, but this time connected to the regulatory region of the *lac* operon. The *lac* promoter is repressed by the *lac* repressor and will be active only in the presence of an inducer. Thus, if we take the second construction alone, gene *cII* will be expressed when, and only when, inducer is present. If we take the first construction alone, we have the vicious circle already described. What will be the behavior of a network including *both* constructions? Starting from an initial situation in which *cII* and inducer are both absent, both genes are silent, the first for lack of *cII* product and the second for lack of inducer. If we add inducer, the second gene will be switched on and will form *cII* product, which will switch on the first gene, producing more *cII* product, and from this point the first gene will remain on, even if inducer is removed (thus switching off the second gene).

The system could be made more interesting if the first copy of the *cII* gene (under *pI* control) carried a mutation making its product thermosensitive. In this case, the system could be

switched off by a brief heat shock, which would inactivate the *cII* product present and "unprime" the pump.

The details are probably easier to follow in a state table. The system can be described by the following graph and naïve logical expressions:



in which c_1 and c_2 represent the levels of the products (and C_1 and C_2 the levels of expression) of the first and second copies of the *cII* gene; I and t represent inducer and temperature, respectively. The state table is

Complete State Table

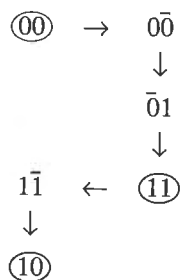
$t = 0, I = 0$		$t = 0, I = 1$		$t = 1, I = 1$		$t = 1, I = 0$	
$c_1 c_2$	$C_1 C_2$	$c_1 c_2$	$C_1 C_2$	$c_1 c_2$	$C_1 C_2$	$c_1 c_2$	$C_1 C_2$
Ⓐ00	00	00̄	01	00̄	01	Ⓐ00	00
00̄1̄	10	00̄1̄	11	00̄1̄	11	00̄1̄	10
1̄1̄	10	Ⓐ1̄1̄	11	Ⓐ1̄1̄	11	1̄1̄	10
Ⓐ10	10	10̄	11	10̄	01	10̄	00

Compact State Table

00	01	11	10	t, I
Ⓐ00	00̄	00̄	Ⓐ00	
00̄1̄	00̄1̄	00̄1̄	00̄1̄	
1̄1̄	Ⓐ1̄1̄	Ⓐ1̄1̄	1̄1̄	
Ⓐ10	10̄	10̄	10̄	

Starting from the stable state Ⓐ00 in the left column (low temperature, no inducer), if we add inducer (shift to the second column), the state 00̄ is reached and the product of the second *cII* gene will appear after a delay t_2 , displacing the system to state 00̄1̄ (line 2). The first *cII* gene is now switched on and will form *cII* product after a delay t_1 (stable state Ⓐ1̄1̄, line 3). If we now remove the inducer (shift back to the first column), state 1̄1̄ is reached, the second

copy of gene *cII* is switched off, and the corresponding product eventually disappears, leading to the stable state $\textcircled{10}$ (line 4), in which only the first *cII* gene is on and only its (thermosensitive) product is present.



This situation is stable. However, if we now expose the system to high temperature (shift to column 4), state $\bar{1}0$ is reached. The thermosensitive product of the first *cII* gene is denatured and no longer ensures the autocatalytic function of the gene. The system stabilizes in state $\textcircled{00}$, in which neither of the two copies of the *cII* gene is expressed, and remains in this state even if it is returned to low temperature.

The system can thus be switched on permanently by a transient signal (adding inducer for a while), and it can be switched off permanently by another signal (raising the temperature for a while). Such devices will no doubt be used in genetic engineering in the future. However, we are convinced that they *are* being used already in naturally occurring developmental processes.

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Chapter 15

MANY STEADY STATES**TABLE OF CONTENTS**

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I. INTRODUCTION

Most theoretical work on multistationarity deals with systems with only a few steady states, typically only three (however, see Reference 1). Higher organisms, on the other hand, contain over 100 distinct cell types. If we hope, not to *explain* them, but merely to *account* for their number in terms of steady states, we must know what types of gene networks are able to generate *many* distinct states.

To the question, "How can one design a system with n steady states?", the usual answer is, "Simply use interactions such that the algebraic degree of the steady-state equations is high enough; after all, an equation of degree n can have up to n real roots." However, although a certain minimum degree is necessary, it is by no means sufficient to guarantee multiple real roots. In particular, we know that a simple negative feedback loop with a sigmoid interaction can have only one steady state and a simple positive loop, no more than three, however high the coefficient n in the Hill functions. As we shall see, there is a simple way to obtain many steady states. For this analysis, the combined use of the logical and differential descriptions turned out to be particularly fruitful.²

II. SYSTEMS WITH SEVERAL INDEPENDENT POSITIVE LOOPS

The simplest system with many steady states is one with several independent positive

loops. Consider the system $x_1 \overset{+}{\curvearrowright}, x_2 \overset{+}{\curvearrowright}, \dots, x_m \overset{+}{\curvearrowright}$, whose generalized kinetic logical description is

$$\begin{aligned} X_1 &= d_{x_1}(K_1 x_1) = K_1 x_1, \\ X_2 &= d_{x_2}(K_2 x_2) = K_2 x_2, \\ &\vdots \\ X_m &= d_{x_m}(K_m x_m) = K_m x_m. \end{aligned}$$

For $K_1 = K_2 = \dots = K_m = 1$, we have

$$\begin{aligned} X_1 &= x_1, \\ X_2 &= x_2, \\ &\vdots \\ X_m &= x_m \end{aligned}$$

in which each of the 2^m logical states is stable. Thus, with m independent positive loops with proper parameter values, we have 2^m stable states.

Consider, now, the one-element loop $x_1 \overset{+}{\curvearrowright}$, whose differential description is

$$\frac{dx_1}{dt} = k_1 F_1^+(x_1, \vartheta) - k_{-1} x_1,$$

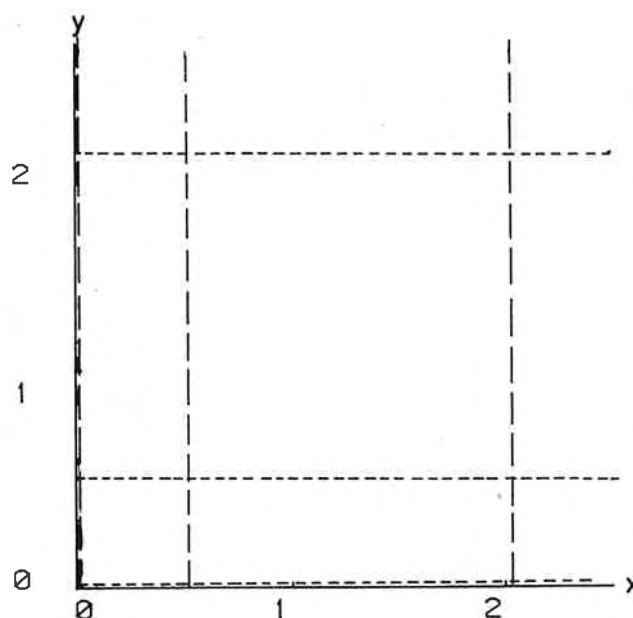


FIGURE 1. Nullclines of the system (1). As usual, the nullcline $dx/dt = 0$ is in long dashes and $dy/dt = 0$ is in short dashes. For clarity, nullclines that lie on an axis are shifted slightly.

We know that, provided $k_1/k_{-1}\vartheta > n/(n-1)^{(n-1)/n}$ (i.e., that the positive loop is “effective”), there are three steady-state values of x_1 , two stable and one unstable (cf. Chapter 12). Since x_1 is regulated only by itself, these values are independent of the other variables. If the sys-

tem includes additional isolated, “effective” positive loops x_2^+ , ..., x_m^+ , each of these variables will also have three steady-state values independent of the other variables. Thus, in a system comprising m independent positive loops, we can have up to 3^m steady states, of which 2^m can be stable.

In Figure 1 are shown the nullclines of the two-variable differential system:

$$\begin{aligned}\frac{dx}{dt} &= \frac{2x^5}{(1+x^5)} - x, \\ \frac{dy}{dt} &= \frac{2y^5}{(1+y^5)} - y.\end{aligned}\tag{1}$$

As could be guessed, each nullcline has three parallel branches, corresponding to the three steady-state values of the variable in question. It can easily be shown that the four intersections involving two “outside” (boundary) branches are stable nodes, the four involving a boundary branch and a “middle” (threshold) branch are saddle points, and the intersection between the two threshold branches is an unstable node.

III. NETWORKS COMPRISING INTERACTING POSITIVE LOOPS

The analysis — logical or differential — of *isolated* loops is trivial since the loops are independent and can be treated separately. In real systems, there are usually interactions among the loops. Differential analysis of such systems is no longer trivial, and the logical analysis is of tremendous help. First, it provides a finite number of qualitatively different patterns of behavior that can be expected from the system. For those considered interesting, it then guides the choice of parameter values for obtaining similar behavioral patterns in the differential description.

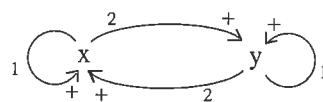
The addition of further interactions usually decreases the number of stable states. In the naïve logical description, adding interactions between loops always results in a decrease of the number of stable states. For example,

$X = x$ $Y = y$	$X = x$ $Y = x + y$	$X = x + y$ $Y = y$	$X = x + y$ $Y = x + y$	$X = x + \bar{y}$ $Y = y$																																																		
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In fact, if we consider a situation in which *every* logical state is stable (as with m independent positive loops), obviously any change in the state table will result in a loss of stable states.

However, the naïve logical description, as we have seen, deals with special cases ($\vartheta_{11} = \vartheta_{21}$, $\vartheta_{12} = \vartheta_{22}$). If we take into account the fact that a variable acting at more than one point has more than one threshold value, it is found that additional interactions can actually increase the number of stable states if additional positive loops are created. Thus, the prediction from generalized kinetic logic is that in a differential system, appropriate monotonic sigmoid interactions among m elements can result in more than 2^m stable states (and, as a matter of fact, in more than 3^m steady states) for m positive loops.

Consider the system described by the graph of interactions



in which the figures indicate the lower and higher thresholds (see Chapter 7). For instance, x acts on itself with its lower threshold and on y with its higher one. The general logical relations are

$$X = d_x(K_{11}^1 x + K_{12}^2 y)$$

$$Y = d_y(K_{21}^2 x + K_{22}^1 y)$$

and the stable state is

$02/K_{12}, K_{22}$	$12/K_{11+12}, K_{22}$	$22/K_{11+12}, K_{21+22}$
$01/0, K_{22}$	$11/K_{11}, K_{22}$	$21/K_{11}, K_{21+22}$
$00/0, 0$	$10/K_{11}, 0$	$20/K_{11}, K_{21}$

We can obtain the four stable states of the naïve description by setting $K_{11} = K_{22} = 1$:

$02/K_{12}, 1$	$12/K_{11+12}, 1$	$22/K_{11+12}, K_{21+22}$
$\textcircled{01}/0, 1$	$\textcircled{11}/1, 1$	$21/1, K_{21+22}$
$\textcircled{00}/0, 0$	$\textcircled{10}/1, 0$	$20/1, K_{21}$

Of the remaining states, $22/K_{11+12}, K_{21+22}$ can be made stable for $K_{11+12} = K_{21+22} = 2$. We then have

$02/-1$	$12/21$	$\textcircled{22}/22$
$\textcircled{01}/01$	$\textcircled{11}/11$	$21/12$
$\textcircled{00}/00$	$\textcircled{10}/10$	$20/1-$

Thus, for these values of the logical parameters, the generalized logical description predicts *five* stable states!

We built a differential system whose parameters respect the above constraints. For $n \rightarrow \infty$ it is easy to draw the nullclines (cf. Chapter 8) and infer from them the number and location of the steady states. In Figure 2 are shown the qualitatively similar nullclines calculated for $n = 20$ and for the parameter values given in the legend. There are 11 intersections between the nullclines $dx/dt = 0$ and $dy/dt = 0$, thus 11 steady states. We expect the steady states at the intersection of two boundary lines to be stable (\bullet); the others are unstable, either saddle points (\square) or unstable nodes (\diamond). All these steady states can, in fact, be identified on logical grounds using the method described in Chapter 8, Section V.

In Table 1, for each steady state we show (1) the values inferred from the discrete analysis, (2) those calculated from the differential analysis for $n = 20$, and (3) the roots of the characteristic equation and the inferred nature of the steady state. *There are, indeed, five stable and six unstable states.*

IV. MORE THAN ONE LOOP FOR ONE VARIABLE?

A gene product may influence its own rate of synthesis by acting at more than one site on the DNA, which could imply more than one threshold concentration. In bacteriophage λ , for example, the repressor product of the cI gene is known to exert a positive effect on its own synthesis at low concentration and a negative effect at high concentration. A case of this type will be treated in Chapter 16, in which we discuss systems comprising both positive and negative loops.

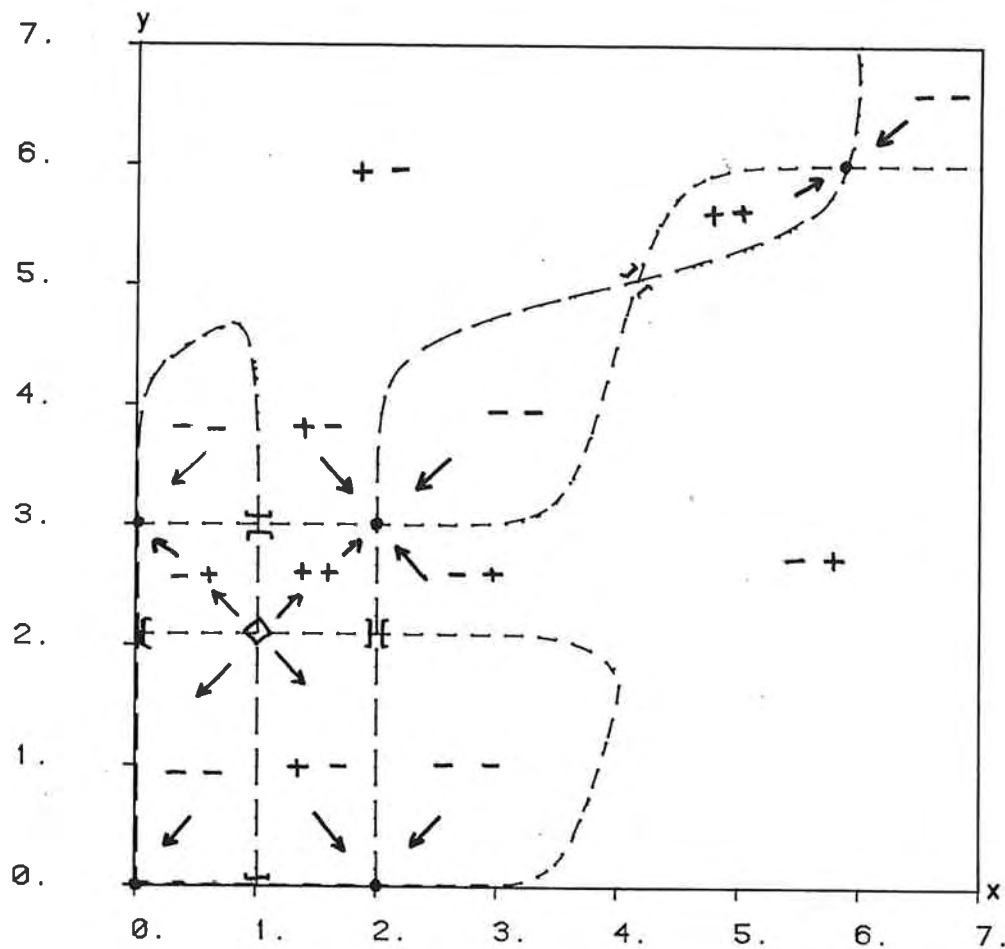
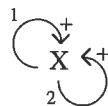


FIGURE 2. Nullclines of a two-variable system with 11 steady states. The parameter values are

$$k_{ij} = \begin{pmatrix} 2 & 4 \\ 3 & 3 \end{pmatrix} \quad k_{-1} = \begin{pmatrix} 1 \\ 1 \end{pmatrix} \quad \phi_{ij} = \begin{pmatrix} 1 & 5 \\ 4 & 2 \end{pmatrix}$$

Here, we will consider the possibility that an element may exert two distinct levels of positive control on its own synthesis:



This type of situation could occur, for example, if a gene product x acts as a transcriptional activator of its own gene X weakly as a monomer (at low concentration) and strongly as an oligomer (at high concentration). This can be described in generalized kinetic logic by the relation:

$$X = d_x(K_1^1 x + K_2^2 x).$$

TABLE 1

Location of Steady State			Nature of Steady State	
General	Particular		Roots of characteristic equation	Inferred nature
	$n \rightarrow \infty$	$n = 20$		
(0, 0)	(0, 0)	(0, 0)	-1, -1	Stable node
$(K_{11}, 0)$	(2, 0)	$(1.99, 2 \times 10^{-5})$	-0.99, -0.99	
$(0, K_{22})$	(0, 3)	$(1.4 \times 10^{-4}, 2.99)$	-0.99, -1	
(K_{11}, K_{22})	(2, 3)	(2.00, 2.99)	-0.99, -0.99	
$(K_{11} + K_{12}, K_{21} + K_{22})$	(6, 6)	(5.89, 5.99)	-0.96, -1.03	
$(\vartheta_{11}, 0)$	(1, 0)	$(1, 2.7 \times 10^{-12})$	9, -1	Saddle point
$(0, \vartheta_{22})$	(0, 2)	$(1.0 \times 10^{-7}, 2.08)$	5.1, -1	
(K_{11}, ϑ_{22})	(2, 2)	(1.99, 2.08)	5.1, -0.9	
(ϑ_{11}, K_{22})	(1, 3)	(0.99, 2.99)	5.1, -0.99	
$(\vartheta_{12}, \vartheta_{21})$	(4, 5)	(4.15, 5.04)	6.05, -8.05	
$(\vartheta_{11}, \vartheta_{22})$	(1, 2)	(0.99, 2.08)	9, 5.1	Unstable node

Setting $d_x(K_1) = K_I$, $d_x(K_2) = K_2$, and $d_x(K_1 + K_2) = K_{I+2}$, the state table is

x	X
0	0
1	K_I
2	K_{I+2}

For $K_I = 1$ and $K_{I+2} = 2$, the state table becomes:

x	X
①	0
①	1
②	2

and there are three stable states.

Let us now examine this as a guide to construct a one-element differential system exhibiting the same behavior, i.e., three stable states. The differential equation is

$$\frac{dx}{dt} = k_1 F^+(x, {}^1\vartheta) + k_2 F^+(x, {}^2\vartheta) - k_{-1}x$$

and the steady state equation is

$$x = (k_1/k_{-1})F^+(x, {}^1\vartheta) + (k_2/k_{-1})F^+(x, {}^2\vartheta) = F(x).$$

From the discrete analysis, we can predict that if k_1/k_{-1} is sufficiently greater than ϑ_1 and smaller than ϑ_2 , and k_2/k_{-1} is sufficiently greater than ϑ_2 , there should be three stable states.

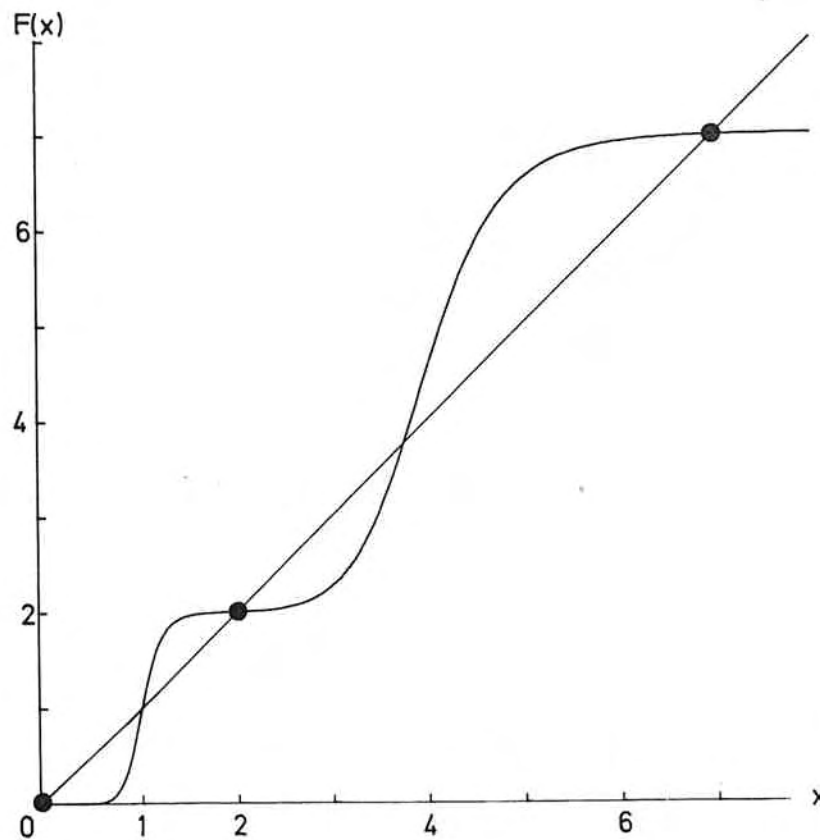


FIGURE 3. A one-variable system with five steady states. The steady-state equation is

$$x = \frac{2x^6}{1 + x^6} + \frac{5x^6}{4^6 + x^6}$$

In Figure 3 is shown a plot of $y = x$ and $y = F(x)$ for parameter values which fit these constraints. There are, indeed, five steady states, three of which are stable.

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1. Kauffman, S. A., Control circuits for determination and transdetermination, *Science*, 181, 310, 1973.
2. Thomas, R. and Richelle, J., Positive feedback loops and multistationarity, *Discrete Appl. Math.*, 19, 381, 1988.

Chapter 16

**SYSTEMS WITH POSITIVE AND NEGATIVE FEEDBACK
LOOPS****TABLE OF CONTENTS**

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I. INTRODUCTION

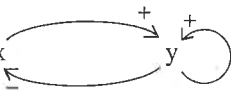
We saw in Chapters 12 and 13 how positive feedback loops generate multiple steady states, and in Chapters 10 and 11 how negative feedback loops generate homeostasis, usually associated with damped or sustained periodicity. In this chapter, we will consider systems which comprise at least one negative and one positive feedback loop and see how they generate both multistationarity and periodicity.

In fact, three- or more-element networks comprising both negative and positive loops can generate much more complex patterns of behavior, including complex periodicity (cf. Appendix 4) and "chaos".¹⁻³ Recent developments in the logical analysis by Snoussi should permit us to present a logical (discrete) description of chaotic systems in the near future. Here, we will focus on the respective roles of the two types of loops in periodicity and multistationarity.

Starting from a negative loop, we have a single steady state which, if the loop is "effective" (cf. Chapter 10), is a *focus*; at least in the case of one- and two-element loops, this focus is always stable. As we will see in Section III, grafting an autocatalytic (positive) loop onto such a negative loop not only can generate multistationarity, but also can destabilize the focus associated with the negative loop, resulting in permanent (undamped) periodicity, even in the two-variable system. This effect has been used extensively to account for stable periodicity in models of two-element systems.^{4-6,8}

II. GRAFTING AN AUTOCATALYTIC (POSITIVE) LOOP ONTO A NEGATIVE LOOP: MULTISTATIONARITY

Let us start with a two-element negative loop and introduce an autocatalytic interaction on one of the elements. In our analysis, we will use both the differential and discrete descriptions. This is legitimate since we know how to find parameter values giving the same qualitative behavior in the two descriptions (cf. Chapter 8), and we can considerably simplify our task by choosing for each point the description that is easier to handle. In fact, the analysis nicely illustrates the complementarity of the two descriptions.

We will analyze the system whose graph of interactions is . The differential equations are

$$dx/dt = k_{12}F^-(y, \vartheta_{12}) - k_{-1}x$$

$$dy/dt = k_{21}F^+(x, \vartheta_{21}) + k_{22}F^+(y, \vartheta_{22}) - k_{-2}y$$

where F^+ and F^- represent positive and negative Hill functions (sigmoid), respectively. If $k_{22} = 0$, we have a simple two-element negative loop. We already know that in this case the system has a single, stable steady state which, for appropriate parameter values (k_{12}/k_{-1} sufficiently greater than ϑ_{21}), is a focus (cf. Chapter 10). Furthermore, this focus should lie near the point $(\vartheta_{21}, \vartheta_{12})$ (cf. Chapter 8).

We now increase the value of k_{22} in order to amplify the contribution of the autocatalytic term. We will first examine the situation with generalized kinetic logic to get a rapid view of the different patterns of behavior we can expect. The logical description of this system, given in another context in Chapter 7, is for $(\vartheta_{12} < \vartheta_{22})$:

$$X = d_x(K_{12}\bar{y})$$

$$Y = d_y(K_{21}x + K_{22}y)$$

in which $K_{12} = k_{12}/k_{-1}$, etc. and d_x and d_y are discretizations in the scales of x and y , respectively (cf. Chapter 7). The state table is

02/0 K_{22}	12/0 $K_{21} + 22$
01/00	11/0 K_{21}
00/ K_{12} 0	10/ $K_{12}K_{21}$

with $K_{12} = d_x(K_{12})$, etc. Since y has two thresholds and x one, X , x , and K_{12} can have the values 0 or 1 and Y , y , K_{21} , K_{22} , and $K_{21} + 22$ can have the values 0, 1, or 2. We assume that the terms describing the negative loop are strong ($K_{12} = 1$ and $K_{21} = 2$), and we examine the effect of increasing the autocatalytic term K_{22} from 0 to 2.

$$K_{22} < 2$$

0 $\bar{2}$ /00	$\bar{1}2/02$
0 $\bar{1}$ /00	$\bar{1}\bar{1}/02$
$\bar{0}0/10$	$\bar{1}\bar{0}/12$

$$K_{22} = 2$$

$\textcircled{02}/02$	$\bar{1}2/02$
0 $\bar{1}$ /00	$\bar{1}\bar{1}/02$
$\bar{0}0/10$	$\bar{1}\bar{0}/12$

It can be seen that for $K_{22} < 2$, there is a periodic attractor. For $K_{22} = 2$, there is a choice between the periodic pattern and a stable state, $\textcircled{02}$. The decision between the two patterns is taken at state $\bar{1}\bar{1}/02$. Which pathway is chosen depends on the relative rates of *decay of x* and *synthesis of y* . We can thus expect that in the differential system, for sufficiently high values of k_{22} (which ensures sufficiently high rates of synthesis of y), the stable pattern will overcome the periodic one. This is indeed found in the differential description. In Figure 1 are plotted the steady-state value(s) of y as a function of k_{22} . It can be seen that for low values of k_{22} , y has a single steady-state value (and, as we shall see, it is a focus). As k_{22} increases, there suddenly appears a second steady state that immediately splits into two steady states. In Thom's language,⁷ this situation, in which the qualitative properties of the system suddenly change, is called a "catastrophe". There is thus a range of values of k_{22} for which y has three steady-state values. As k_{22} increases further, the middle and upper values move closer, until at high values of k_{22} , the latter fuse and disappear, leaving only the upper steady state.

III. GRAFTING AN AUTOCATALYTIC (POSITIVE) LOOP ONTO A NEGATIVE LOOP: DESTABILIZATION OF THE FOCUS

It is interesting to take the analysis further and ascertain the nature of the steady states for different values of k_{22} . This is most easily done by finding the roots of the characteristic equation for each value of k_{22} , then plotting the product of the roots (P) vs. the sum (S) for each point. The location of a point in S - P space, as described in Appendix 3, immediately reveals the nature of the corresponding steady state. For points located in the "south", we have $P < 0$. The roots are thus real and of opposite sign, meaning the steady state is a saddle point. Points located in the NW quadrant ($P > 0$, $S < 0$) have both roots (or their real part) negative, meaning the steady states are stable, whereas points located NE ($P > 0$, $S > 0$)

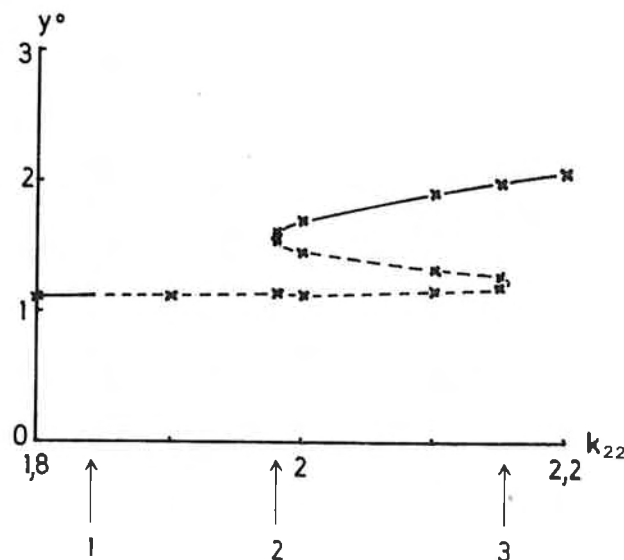


FIGURE 1. The steady-state values of variable y for increasing values of the parameter k_{22} . The parameter values chosen are

$$n = 5 \quad k_{ij} = \begin{pmatrix} - & 2 \\ 2 & k_{22} \end{pmatrix} \quad k_{-1} = \begin{pmatrix} 1 \\ 1 \end{pmatrix} \quad \vartheta_{ij} = \begin{pmatrix} - & 1 \\ 1 & 1.2 \end{pmatrix}$$

Thus, for the logical parameters we have $K_{12} = 1$ (i.e., $k_{12}/k_{-1} > \vartheta_{21}$) and $K_{21} = 2$ (i.e., $k_{21}/k_{-2} > \vartheta_{22}$); K_{22} will vary according to the value of k_{22} . The curve is drawn solid or dotted, according to whether the steady state is stable or unstable (see Section III). Arrows indicate the critical values of k_{22} : (1) the unique steady state, a focus, becomes unstable; (2) additional steady states appear; and (3) the original steady state fuses with one of the new ones and the two disappear.

have both roots (or their real part) positive, indicating unstable steady states. Finally, points lying above the parabola $S^2 - 4P = 0$ represent foci, whereas points between the parabola and the S axis represent nodes. These regions are shown in Figure 2.

In our present example, for low values of k_{22} , the points lie in the NW quadrant, above the parabola (Figure 2), indicating that in this range the (unique) steady state is a stable focus (the characteristic equation has complex roots with a negative real part). As k_{22} increases, the point representing the steady states moves toward the SE. When it crosses the P axis, the real part of the roots becomes positive. At this point, the system will bifurcate toward a limit cycle solution—a “Hopf bifurcation”. For each value of k_{22} in this range, the (unique) steady state is an unstable focus. **Thus, a sufficiently strong autocatalytic term can destabilize the focus.** As k_{22} increases further, we reach a second critical value: for $k_{22} \approx 1.98$, a second steady state appears on the negative part of the S axis and immediately splits into two steady states, one above and one below the S axis, and their distance increases with increasing k_{22} . The first, in the NW quadrant, represents a stable node, and the second, in the south ($P < 0$), represents a saddle point. Finally, as k_{22} increases yet further, the points representing the saddle point and the original steady state meet on the S axis and disappear. Before this happens, it can be seen that the original steady state passes from an unstable focus (imaginary roots) to an unstable node (real roots) as the point crosses the parabola.

Let us now reexamine the destabilization of the focus. As shown in Chapter 6 and Appendix 3, for a simple two-element negative loop (which is our situation for $k_{22} = 0$) it is

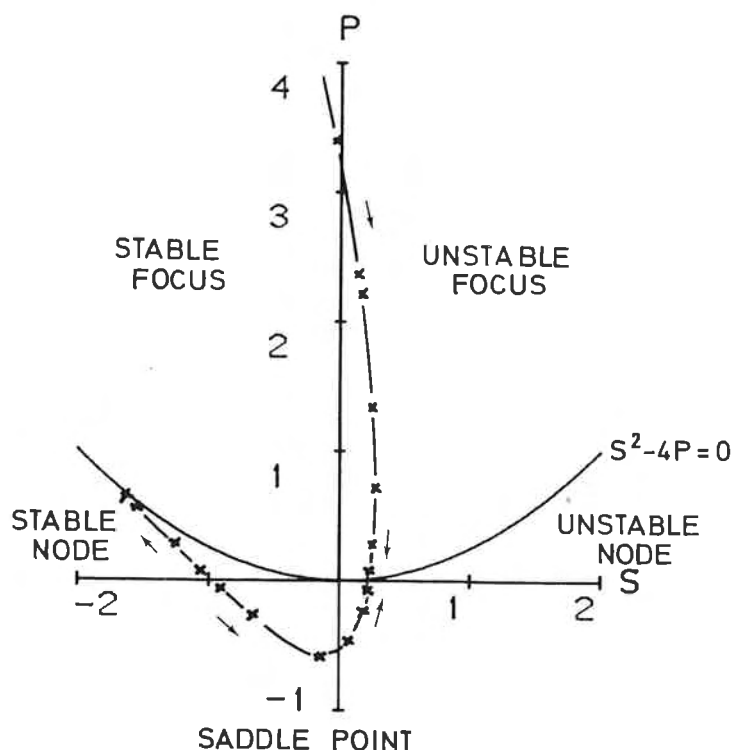


FIGURE 2. The stability of the steady states for increasing values of k_{22} . The parameter values are the same as in Figure 1.

necessarily stable because the real part of the roots of the characteristic equation is equal to $(a_{11} + a_{22})/2$, or $(-k_{-1} - k_{-2})/2$, which is negative. However, if there is an autocatalytic term $k_{22}F^+(y)$, $a_{11} + a_{22}$ becomes $-k_{-1} - k_{-2} + k_{22}[dF^+(y, \vartheta_{22})/dy]_0$. The third term, which is positive, will make the sum positive if it exceeds $k_{-1} + k_{-2}$. In the case of sigmoid functions, $dF^+(y, \vartheta_{22})/dy$ is a bell-shaped function whose value is near zero except in the vicinity of the inflection point y_1 of F^+ , itself close to the threshold ϑ_{22} (see Figure 3).

In our system, the focus is located near $(\vartheta_{21}, \vartheta_{12})$; in other words, $y^0 \approx \vartheta_{12}$. But we have just seen that $k_{22}[dF^+(y, \vartheta_{22})/dy]_0$ is very small unless y^0 is close to the inflection point of $F^+(y)$, which lies near ϑ_{22} . Thus, we do not expect the autocatalytic term to destabilize the focus unless the two thresholds of y , ϑ_{12} and ϑ_{22} , are close to each other, and this constraint becomes stronger as the sigmoid $F^+(y)$ becomes steeper.

This is illustrated in Table 1, in which we compare the nature of the steady state for increasing values of the exponent n in the Hill functions, holding the other parameters constant. In the system considered, the steady state is a focus that is stable for $n = 4$, unstable for $n = 5$ to 13 , and stable again for $n \geq 14$.

In the special case $\vartheta_{12} = \vartheta_{22}$, this problem disappears; the higher n , the higher $k_{22}[dF^+(y)/dy]_0$, and unstable foci are readily found. This was the situation in Case 11 analyzed in Chapter 8. There, the focus was the only steady state, giving rise to a limit cycle when unstable.

IV. THE TWO TYPES OF DESTABILIZATION HAVE DIFFERENT MECHANISMS

As pointed out in Chapter 6, multiplying all the k 's and k_{-} 's of a differential equation by the same factor will change the timing of the process, but will not affect the steady-state

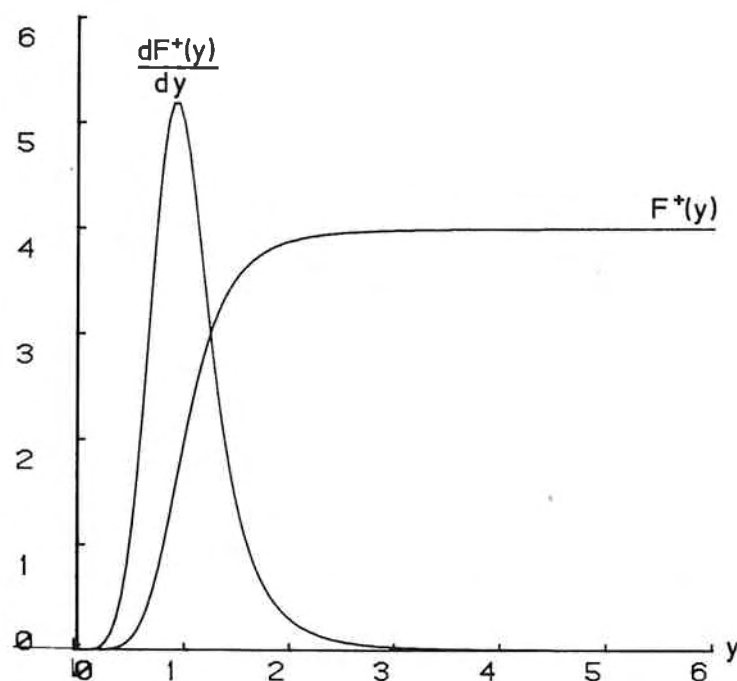


FIGURE 3. The functions $dF^+(y)/dy$ and $F^+(y)$. The maximum of $dF^+(y)/dy$ corresponds to the inflection point y_i of $F^+(y)$. The point y_i is less than the threshold value ϑ_{22} ($\vartheta_{22} = [(n+1)/n - 1]^{1/n} y_i$), but the steeper the sigmoid (i.e., the higher the Hill exponent n), the closer the two points will be. It is also clear that the steeper the sigmoid is, the higher and narrower the derivative curve will be.

TABLE 1

Hill number	Location of the focus	S	P	$S^2 - 4P$	ω	Stability of the focus
$n=4$	$x = 0.463$ $y = 1.349$	-0.56	0.329	-1.00	$-0.28 \pm 0.5i$	Stable
$n=5$	$x = 0.669$ $y = 1.147$	0.20	1.80	-7.24	$0.1 \pm 1.3i$	Unstable
$n=13$	$x = 0.972$ $y = 1.00$	0.176	40	-162	$0.085 \pm 6.3i$	Unstable
$n=14$	$x = 0.978$ $y = 1.00$	-0.010	47.7	-191	$-0.005 \pm 6.9i$	Stable
$n=20$	$x = 0.994$ $y = 1.00$	-0.97	100	-399	$-0.48 \pm 9.9i$	Stable

Note: The system is described in Figures 1 and 2. Parameter values are

$$k = \begin{pmatrix} - & 2 \\ 2 & 2.05 \end{pmatrix} \quad k_- = \begin{pmatrix} 1 \\ 1 \end{pmatrix} \quad \vartheta = \begin{pmatrix} - & 1 \\ 1 & 1.2 \end{pmatrix}$$

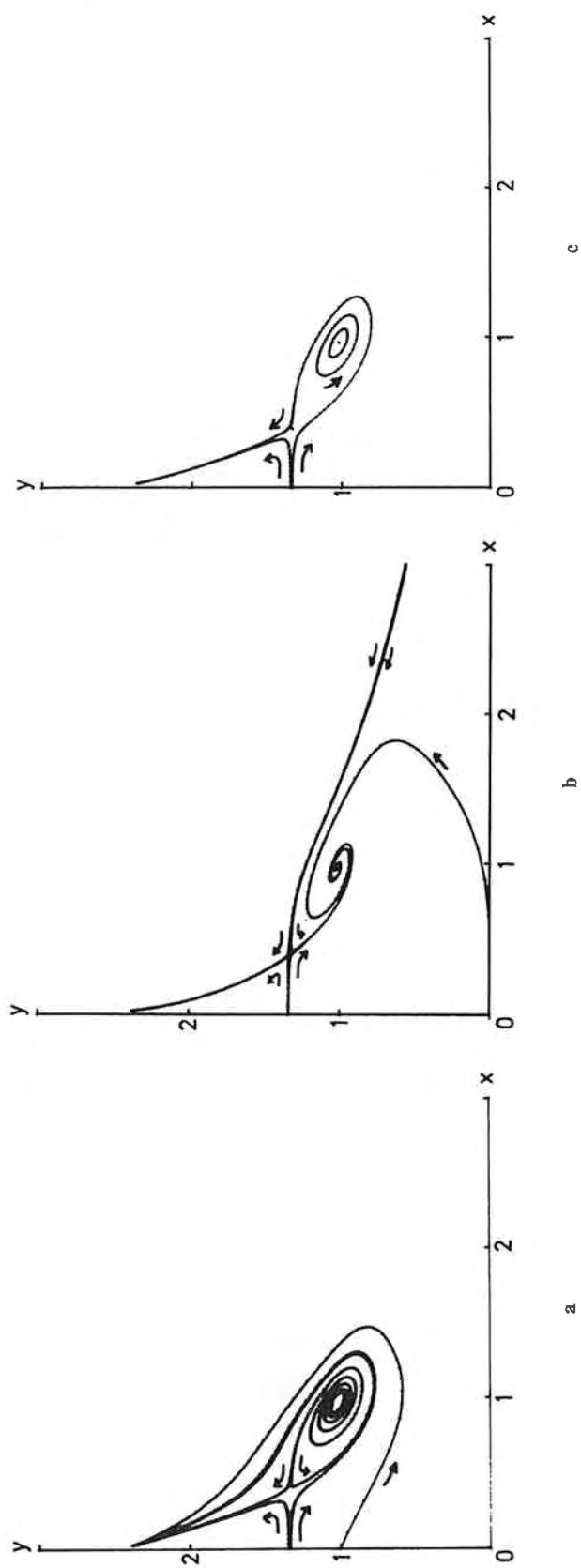


FIGURE 4. Stability of a focus as a function of the kinetic parameters. We use the same system as in Figure 1, with the following sets of parameter values. Note that the k_{ij}/k_{-i} ratios are the same in all three cases.

$$(a) \quad n=5, \quad k_{ij} = \begin{pmatrix} - & 2 \\ 1 & 2.5 \end{pmatrix}, \quad k_{-1} = \begin{pmatrix} 1 \\ 1 \end{pmatrix}, \quad \phi_{ij} = \begin{pmatrix} - & 1 \\ 1 & 1.3 \end{pmatrix}$$

$$(b) \quad n=5, \quad k_{ij} = \begin{pmatrix} - & 4 \\ 1 & 2.5 \end{pmatrix}, \quad k_{-1} = \begin{pmatrix} 2 \\ 1 \end{pmatrix}, \quad \phi_{ij} = \begin{pmatrix} - & 1 \\ 1 & 1.3 \end{pmatrix}$$

$$(c) \quad n=5, \quad k_{ij} = \begin{pmatrix} - & 2.2934 \\ 1 & 2.5 \end{pmatrix}, \quad k_{-1} = \begin{pmatrix} 1.1467 \\ 1 \end{pmatrix}, \quad \phi_{ij} = \begin{pmatrix} - & 1 \\ 1 & 1.3 \end{pmatrix}$$

equations since the K 's (i.e., the ratio k/k_-) remain unchanged. Thus the nullclines and steady-state values do not change, but the stability of a focus can be affected. This is illustrated in Figure 4, in which the unstable focus (panel a) is stabilized simply by doubling k_{12} and k_{-1} (panel b). Note that although the focus is unstable in panel a, instead of generating a limit cycle, it leads to the stable state of the system. If the multiplicative factor is chosen such that for the "lower" steady state the roots of the characteristic equation are pure imaginary ($S = 0$), the steady state is neither attractive nor repulsive, and the trajectories form closed curves around it (panel c). Such a steady state is called a *center*.

Thus, the steady-state equations and, consequently, the nullclines tell us everything about the number and location of the steady states, but not about the stability of a focus. In this respect, the steady-state equations resemble our logical relations, which distinguish clearly between, say, a node (stable state) and a focus (cycle), but do not differentiate stable and unstable foci.

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Part III: Applications



Chapter 17

**EPIGENETIC DIFFERENCES AND MULTIPLE
STEADY STATES****TABLE OF CONTENTS**

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I. ANTIGENIC VARIATION IN *PARAMECIUM AURELIA*

In the summer of 1948, the French Centre National de la Recherche Scientifique organized an international colloquium in Paris entitled "Biological Units Endowed with Genetic Continuity".¹ It was an exciting period for biologists. DNA had been identified a few years earlier as the *Pneumococcus* transforming principle but no one could say to what extent it carried the genetic information involved in nuclear and cytoplasmic inheritance. One major concern at the meeting, as the title suggests, was with autonomous cytoplasmic particles such as chloroplasts, kinetosomes, or the Kappa factor. Called "plasmagenes", these particles were known to conserve their properties independently of the nuclear genotype. There were several clear cases of cytoplasmic (non-Mendelian) inheritance correlated with cytologically observable entities. However, plasmagenes were also postulated, in a vaguer way, to explain a number of other cytoplasmically determined phenomena, even though they were not associated with any visible particle.

Sonneborn and Beale² presented observations of this type on antigenic variation in *Paramecium aurelia*. A given culture stably produced a specific surface antigen, but could segregate variants expressing another surface antigen. In crosses between different variants, the progeny all showed the "maternal" antigenic type, which was therefore postulated to be determined by a plasmagene. Each line of *P. aurelia* had its own repertoire of potential antigenic states. In a given culture, only one type was expressed, but limited treatment with the corresponding antiserum could cause up to 90% of the cells to express a different antigen of the repertoire. On the other hand, in crosses between independent lines, they found that the *potential* to produce a certain antigen exhibited Mendelian inheritance and thus was determined by a nuclear gene. This posed a paradox: the antigen actually being expressed in a culture was determined by cytoplasmic factors, whereas the potential ability to express it was determined by the nucleus. Sonneborn and Beale suggested that the nuclear genes determined the formation of the various plasmagenes, which then became essentially autonomous unless the antigen whose synthesis they directed were inactivated, e.g., by antiserum.

In the discussion following Beale's talk, Delbrück³ pointed out that these observations could equally well be explained by the existence of multiple steady states, without postulating the presence of plasmagenes. Forty years later, it seems that antigenic variation is indeed epigenetic.¹¹ The cytoplasmic factors that determine the antigenic type being expressed (and exhibit "maternal" inheritance) are presumably regulators of the nuclear genes that code for the various antigens. The action of these regulators results in the expression of only the appropriate gene.

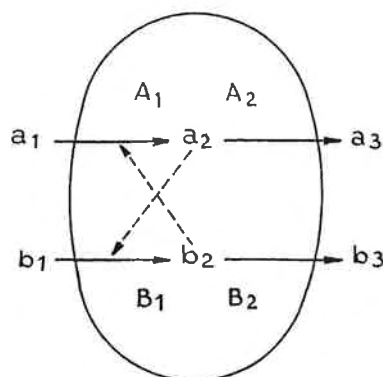
M. Delbrück's remark was so clearly formulated that we reproduce it here (our translation) in its entirety, both for historical interest and as a concise presentation of epigenetic regulation.

"In his discussion of the phenomena observed by Sonneborn and himself, Dr. Beale proposed considering that these phenomena result from the properties of a population of plasmagenes whose reproduction is favored or inhibited by the medium.

I do not intend to contest this conception but would like to draw attention to certain general properties of systems in so-called "steady state", properties which must be taken into consideration before postulating the existence of biological units endowed with genetic continuity in any or all cases in which the genetic continuity of a function is observed.

The argument I wish to develop is the following: *many systems in steady state can exhibit several different stable states under identical conditions. They can be shifted from one stable state to another by transient perturbations.*

This general proposition can be illustrated by a simple model. In the following diagram (Figure 1), the letters A₁, A₂, B₁, and B₂ stand for different enzymes in a cell, which is represented by the circle. The letters a₁, b₁ stand for substances in the medium. Via the action of A₁ and B₁, respectively, these substances are transformed into



intermediate metabolites a_2 and b_2 . The latter, in turn, are the substrates of enzymes A_2 and B_2 , which transform them into waste products a_3 and b_3 . If the medium is constant, the cell will quickly reach a steady state characterized by a certain constant concentration of the intermediate products a_2 and b_2 . In this model, there is only one stable state, determined by the medium and the cell's enzymatic properties.

Let us now add the hypothesis that there exist mutual interactions between the two series of enzymatic reactions. Explicitly, let us suppose that the metabolite a_2 affects the reaction catalyzed by enzyme B_1 such that at *high* concentrations of a_2 this reaction is inhibited.* We further postulate a similar effect of metabolite b_2 on enzyme A_1 . These interactions are shown by dotted arrows in the diagram.

In this new model, it is still true that under constant conditions the cell will reach a steady state. However, there now exist three possible steady states for the same culture conditions, two stable and one unstable. Let us consider, for example, conditions in which substances a_1 and b_1 are at equal concentration. The steady state ultimately reached will depend on the order in which these substances were added to the medium. According to the conditions, the steady state will be characterized by:

- a. Much a_2 , little b_2 , if a_1 was added first. This steady state is stable; we will call it state a .
- b. Little a_2 , much b_2 , if b_1 was added first. This steady state is also stable. We will call it state b .
- c. Equal low concentrations of a_2 and b_2 if the two substances were added simultaneously in equal quantities. This is a steady state, but it is an unstable state in which weak perturbations will cause a shift to state a or to state b .

The shift from state a to state b could be caused by strong *transient* perturbations. For example, if the initial state is a , a *temporary* interruption of the inhibition of B_1 by a_2 will cause a shift from state a to state b .

These alterations could occur via diverse mechanisms: *transient* treatment with anti- a_2 serum, *transient* change of temperature such that the activity of enzyme A_1 is selectively reduced, or *transient* transfer to a medium lacking substance a_1 .

In summary, our model cell can exist in two functionally different steady states without this implying any change in the properties of the genes, plasmagenes, enzymes, or any other structural units. Shifts from one state to another can be caused by *transient* modifications in the medium.

Models of this type can be modified *ad infinitum* to account for a large number of different steady states endowed with any degree of stability. Shifts from one to another could, according to the case, be reversible or irreversible, as in differentiation, where the existence of plasmagenes has also been invoked, without any concrete proof.

I do not claim to propose a theory here explaining the phenomena described by Sonneborn and Beale. I simply wish to insist on the fact that, for systems in steady state (but not for systems at equilibrium), one can envisage diverse explanations of this type which from a general point of view are by no means outlandish or even improbable. The above proposition is not new, and many biologists have a fairly clear idea of what it implies. I thought this simple model would help illustrate and clarify the idea."

II. EPIGENETIC CHANGE IN THE *ESCHERICHIA COLI lac* OPERON

Delbrück's historic comment was a prelude for an entirely new way of looking at differentiation. Soon afterward, his ideas were borne out by experimental work. It was known from studies by Monod and co-workers that the bacterium *Escherichia coli* has the genes required

* Such a property could be due to reversible dimerization of a_2 , with only the dimer being able to inhibit the reaction catalyzed by B_1 .

to utilize the sugar lactose, but that these genes are expressed only in the presence of an "inducer". The natural inducer is a close derivative of lactose itself (produced by the cell from lactose), but there are a number of synthetic analogues that are also good inducers. Some of these cannot be metabolized by the cell and are called "gratuitous" inducers. The enzymes involved are β -galactosidase, which splits the disaccharide lactose into glucose and galactose, and β -galactoside permease, which sits in the membrane and actively pumps lactose and its analogues into the cell from the outside medium. The level of these enzymes is essentially nil in the absence of inducer, but becomes significant within minutes after the addition of inducer. A crucial observation was that in the presence of low external inducer concentrations, a cell that was already induced ("preinduced") would remain induced indefinitely, whereas a cell that was not induced would remain uninduced. We will call this range of inducer concentration a "maintenance" concentration.

This was the starting point for two admirable, complementary series of experiments by Novick and Weiner⁴ and by Cohn and Horibata.⁵ In simplified terms, one can describe their experiments as follows. Take an uninduced culture, add a high concentration of inducer, split the culture into two parts, and dilute them so that the inducer concentration falls to the "maintenance" range. This dilution is made immediately for subculture A, but only after 10 min for subculture B. Knowing the phenomenon of preinduction, one can predict the result: culture A was not exposed to a high inducer concentration (except for a few seconds) and thus remains uninduced, whereas culture B, exposed to a high inducer concentration for 10 min, was induced and will remain so since the residual inducer concentration suffices to maintain the induced state. This is exactly what was observed experimentally. What is absolutely striking is that the two cultures can be diluted indefinitely (more than 130 generations) in the same medium (containing a maintenance concentration of inducer) without changing their state: the subcultures derived from culture A remain uninduced (essentially, no β -galactosidase synthesis), whereas those derived from culture B remain induced (high level of β -galactosidase synthesis). Furthermore, it is readily shown that the populations have not changed genetically: if inducer is removed, all cells become uninduced, and if a high concentration of inducer is added, all cells are rapidly induced.

The essential logical mechanisms underlying these experiments were fully understood in the late 1950s by their authors. Internal inducer is required for the synthesis of permease, but unless the external inducer concentration is high, permease is required to build up a significant internal concentration. Thus, at low external inducer concentrations, we have a vicious circle: internal inducer is required for the synthesis of permease and permease is required for the internalization of inducer. A very simple logical formalization is given in Table 1. Clearly, there is a range of external inducer concentration (the maintenance range) in which *permease behaves autocatalytically* — a typical positive feedback loop — and this is the basis for the two distinct, heritable phenotypes observed.

In the above experiments, we had two cultures of genetically identical bacteria growing in identical environments, yet presenting a heritable phenotypic difference: one had a high level of β -galactosidase synthesis, the other, essentially none. An outside observer unaware of the history of the two cultures could easily conclude that the bacteria were genetically different. The formal analogy with the antigenic variation in *P. aurelia* discussed by Delbrück is obvious: there are two stable states, and the cells can be made to pass durably from one to the other by transient perturbations (temporary exposure to high or low inducer concentration). This is a perfectly unambiguous case of an *epigenetic difference*, the first to be clearly established and understood. It remains perhaps the simplest and most elegant illustration of an epigenetic change.

TABLE 1

The presence and synthesis of permease are represented by the Boolean variable and function y and Y , respectively, and the external inducer concentration by I . We consider three levels of external inducer:

$I = 0$, negligible;

$I = 1$, maintenance;

$I = 2$, inducing.

We use the Boolean variables 1I and 2I : $^1I = 0$ iff $I < 1$, and $^2I = 0$ iff $I < 2$. The logical relation is

$$Y = ^2I + ^1Iy.$$

It simply says that permease is synthesized if there is a high external inducer concentration or if there is a maintenance inducer concentration and permease is already present. In the state table, we treat I as an input variable.

$I = 0$		$I = 1$		$I = 2$	
y	Y	y	Y	y	Y
0	0	0	0	0	1
1	0	1	1	1	1
$(Y = 0)$		$(Y = y)$		$(Y = 1)$	

^a A differential treatment of this system can be found in Reference 10.

III. EPIGENETIC MODELS OF MONOD AND JACOB

The very basis of today's ideas on gene regulation is the recognition that there exist regulatory genes whose products, in response to external stimuli, influence the expression of other genes by interacting with specific DNA sequences near the genes to be regulated.⁶ In isolation, these elementary pieces of the regulatory puzzle permit reversible modulation of gene expression by environmental variables. As pointed out in Chapter 9, they form individual positive or negative controls, not feedback loops. However, as soon as Monod and Jacob discovered gene regulators, they began to speculate about the possible effects of looped sets of such elements.⁷ This remarkable paper should be quoted *in extenso*. Here, we will simply show two of the models.

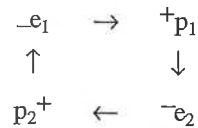
These authors envisaged a system in which a gene E , negatively regulated by a repressor, codes for an enzyme e that catalyzes the synthesis of a product p which, in turn, antagonizes the repressor; in other words, enzyme e produces its own inducer. The system is readily formalized:

$$\begin{aligned} E &= p, \\ P &= e, \end{aligned}$$

ep	EP
00	00
01	10
11	11
10	01

confirming that the system does indeed constitute a positive loop, with two stable states: both enzyme and inducer present ($\textcircled{11}$) or both absent ($\textcircled{00}$).

A second example involves two genes, E_1 and E_2 , coding for enzymes e_1 and e_2 , which catalyze the formation of products p_1 and p_2 , respectively. Product p_1 is a corepressor of gene E_2 and p_2 is corepressor of E_1 . Again, the system forms a positive loop:



The logical relations are

$$P_1 = e_1$$

$$P_2 = e_2$$

$$E_1 = \bar{p}_2$$

$$E_2 = \bar{p}_1$$

and the state table is

$p_1 e_1 p_2 e_2$	$P_1 E_1 P_2 E_2$	$p_1 e_1 p_2 e_2$	$P_1 E_1 P_2 E_2$
0000	0101	$\textcircled{1100}$	1100
0001	0111	1101	1110
$\textcircled{0011}$	0011	1111	1010
0010	0001	1110	1000
0110	1001	1010	0000
0111	1011	1011	0010
0101	1111	1001	0110
0100	1101	1000	0100

The two stable states are $\textcircled{0011}$ (only E_2 and p_2 are present) and $\textcircled{1100}$ (only E_1 and p_1 are present).

These and other examples were presented in 1961 as simple illustrations of the type of logical circuit capable of giving rise to the epigenetic changes that characterize differentiation. In all cases, a simple positive feedback loop was involved.

IV. THE CI-CRO SYSTEM OF BACTERIOPHAGE λ

This system will be described in more detail in Chapter 20 as part of the λ circuitry. Here, we will briefly describe the essential aspects of repressor regulation, elucidated by the Jacob group.⁸

Bacteria lysogenic for λ carry the phage DNA inserted in their own chromosome, where it is called a "prophage". The prophage cI gene directs the synthesis of a repressor, which directly or indirectly represses the transcription of other prophage genes. In particular, the genes coding for phage production and lytic growth are silent. The lysogenic bacterium is thus not inconvenienced by its prophage and, in fact, thanks to the repressor, is immune to infection by other λ phage. The cI gene is negatively regulated by the product of the cro

gene, which, in turn, is repressed by cI . If the λ repressor is temporarily inactivated, the situation quickly becomes irreversible. In the absence of active cI repressor, the cro product is synthesized and, once a threshold concentration is reached, it prevents the synthesis of new cI . Thus, *transient* inactivation of cI repressor can result in a *permanent* loss of immunity. This normally leads to lytic development of the phage and death of the cell. However, if the viral functions responsible for cell death are mutationally inactivated, lysogens can survive despite the loss of immunity. These lysogens can grow indefinitely in either of two stable states, immune or non-immune. This is, again, a beautiful example of an epigenetic difference.

The cI - cro system of phage λ has been elegantly exploited by Toman et al.⁹, who replaced the genes distal to cro by the bacterial gal genes, coding for the enzymes involved in galactose utilization. In this way, cells in the " cro " (nonimmune) phase express the gal genes and are able to metabolize galactose, whereas cells in the " cI " (immune) phase do not express these genes and are unable to use galactose. Using appropriate "indicator" plates, the strain produces red or white colonies, according to whether the cells are in the " cro " or " cI " phase. (Indicator plates contain galactose and a pH indicator; colonies in which the galactose is fermented cause local acidification, producing a red color.)

This sophisticated bacterial strain has interesting properties. If, for example, it is exposed to UV light, the DNA will be damaged and the SOS response will be induced (cf. Chapter 19). One manifestation of SOS induction is the proteolytic cleavage of the λ cI repressor. This leads to a stable conversion of bacteria from the immune state (Gal^- , white colonies) to the nonimmune state (Gal^+ , red colonies). The frequency of red colonies in the irradiated culture is a direct and highly sensitive measure of the degree of DNA damage. UV doses that have no effect on cell viability can cause a significant increase in the frequency of red colonies. The same strain can be — and, in fact, is — used to evaluate DNA damage caused by other agents.

It is well known that UV irradiation and other DNA damaging treatments are mutagenic. In the experiment just described, the UV light could mutationally inactivate the cI gene and, of course, this would also result in conversion from the immune state to the nonimmune state. The mechanism, however, is completely different: SOS induction involves an epigenetic change, whereas mutational inactivation is a genetic change. In fact, the two types of events can be distinguished on the indicator plates. The epigenetic Gal^+ colonies are a paler red since the frequency of recovery of the immune (Gal^-) state is about 10^{-4} , whereas genetic Gal^+ colonies generally contain few, if any, Gal^- revertants and are thus dark red. In the case of UV irradiation, the epigenetic effect is about 100-fold higher than the genetic effect. Other mutagens behave differently. For example, for the powerful mutagen nitrosoguanidine, the genetic effect is more pronounced than the epigenetic effect, and ethylmethanesulfonate produces only the genetic effect.

V. AND SCRAPIE?

Scrapie is a transmissible, mortal disease in sheep that presents a curious paradox: the infectious agent of the disease, isolated from infected animals, contains no nucleic acid!. In fact, the active principle seems to be protein. Of course, a self-propagating protein would violate the paradigm, based on more than 40 years of intensive molecular biology research, that all genetic information is carried by the nucleic acids, DNA and RNA.

A simple hypothesis accounting for this paradox (and respecting orthodoxy) is that the gene coding for the scrapie protein is part of the normal sheep genome. How, then, can we explain that sheep are healthy unless infected by the scrapie protein? The reader will no doubt have guessed: it is sufficient to postulate an autocatalytic positive loop such that the scrapie gene can only be expressed if the scrapie protein is already present (much like lactose per-

mease in the conditions described in Section III above). This type of circuit will have the two stable states "gene off, scrapie protein absent", the normal, healthy state, and "gene on, scrapie protein present", the situation after infection by scrapie protein.

Such a hypothesis, although gratuitous at present, is testable and, if borne out by experiments, would provide a framework for disease control and for the development of resistant lines of sheep. It would also pose an interesting question: what is the normal role of this potentially lethal protein?

The examples of the *E. coli lac* operon and the cI-cro system of λ (Sections II and IV) are particularly striking because the underlying molecular mechanisms of the epigenetic changes are thoroughly understood and, indeed, form positive feedback loops. It seems virtually certain that other, more complex situations in which multiple stable states occur, such as antigenetic variation and possibly scrapie (Sections I and V) will similarly turn out to be based on positive feedback loops. In fact, we are convinced that this type of logical circuit will provide the ultimate explanation for the many stable states reached in the course of embryonic development.

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Chapter 18

LOGICAL TREATMENT OF DIFFUSION**M. Kaufman****TABLE OF CONTENTS**

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I. INTRODUCTION

So far, space — and hence the transport of matter — have not been considered explicitly. The aim of this chapter is to illustrate how diffusion processes can be incorporated into the logical description.

The systems we shall deal with consist of groups of cells communicating via diffusion of the reactants. The cells are in a well-defined environment, and in each of them regulatory processes take place. We shall be interested in the collective behavior arising from the interaction of three processes: (1) mutual mass exchanges, (2) nonlinear chemical kinetics, and (3) exchanges with the surrounding medium.

Simple diffusion is usually described by linear terms that cannot be approximated by step functions or Boolean variables. We shall show in this chapter that this difficulty can be overcome, at least for the determination of the stationary solutions, by introducing a trick to remove the undesired linear terms. Regulatory processes coupled to mass transfer are then readily handled with the generalized logical method. The identification of the stable states and their number simply amounts to evaluating a set of logical parameters and ordering them by magnitude.

Several authors have studied multicellular systems of the kind described here using classical continuous analysis.¹⁻⁷ The occurrence of multiple steady states, some of which correspond to stable spatial patterns, has been demonstrated theoretically and experimentally.⁷ The interest of the logical approach is to uncover all the potentialities that such systems may present without requiring lengthy calculations.

Compartmentalized systems may be considered to model a tissue of initially equipotent cells, each, for instance, with the same genetic circuit. The emergence of a stable partitioning of the tissue into regions with differential genetic expression might be crucial for many differentiation or developmental processes.

We will focus here on the elementary example of two identical interconnected cells containing a simple one-element positive loop. Situations involving more cells or more complex regulation are described elsewhere.⁸

II. A TWO-CELL SYSTEM: DIFFERENTIAL FORMULATION

For illustrative purposes, we shall consider here a simple two-cell system (Figure 1). In each cell, the same autocatalytic process takes place, conveniently described by the one-element positive loop (see Chapters 12 and 13):



The two compartments (I and II), containing homogeneous solutions of product x at concentrations x_1 and x_2 are separated by a thin inactive membrane. There is no reaction within the membrane, which only allows the diffusion of x between the two compartments, from regions of high concentration to regions of low concentration. Moreover, each compartment is in contact with a well-mixed reservoir with a fixed concentration of x , x_{01} and x_{02} .

In the absence of diffusional coupling between the compartments (i.e., if the membrane is impermeable to x), each cell behaves independently, and the time evolution of the concentration of x in each compartment is the result of (1) its exchange with the reservoir:

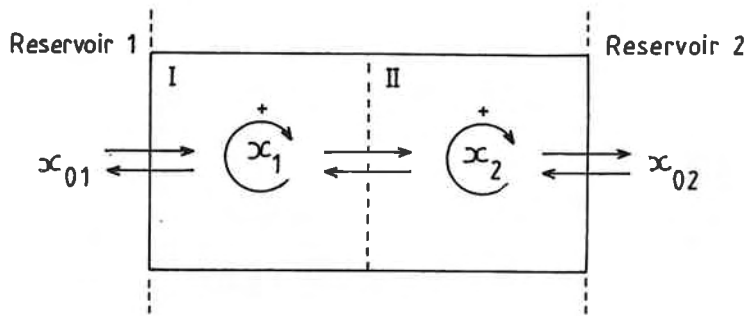


FIGURE 1. A two-cell system.

$$\left(\frac{dx_i}{d\Gamma}\right)_{\text{flow}} = \frac{D}{V_c} (x_{0i} - x_i)$$

where x_{0i} is the constant concentration of x in the reservoir, D is the flow rate (volume per unit time), and V_c is the volume of the cell; and (2) its autocatalytic synthesis (see Chapter 12, Section II):

$$\left(\frac{dx_i}{d\Gamma}\right)_{\text{reaction}} = k \frac{x_i^n}{\theta_x^n + x_i^n}$$

Thus, the equation governing the evolution of x_i in a single cell fed from an external reservoir is given by:

$$\frac{dx_i}{d\Gamma} = \left(\frac{dx_i}{d\Gamma}\right)_{\text{flow}} + \left(\frac{dx_i}{d\Gamma}\right)_{\text{reaction}}$$

$$\rho = \frac{D}{V_c} (x_{0i} - x_i) + k \frac{x_i^n}{\theta_x^n + x_i^n}$$

Substituting the dimensionless time variable $t = T \cdot \frac{D}{V_c}$, which measures time in units of the "characteristic time" $\frac{V_c}{D}$ (the time to "fill" the cell), we obtain:

$$\frac{dx_i}{dt} = x_{0i} - x_i + \sigma \frac{x_i^n}{\theta_x^n + x_i^n} \quad (1)$$

with $\sigma = k \cdot \frac{V_c}{D}$. The parameter σ , whose dimensions are those of x (concentration units), represents the strength of the autocatalytic term.

This equation is similar to that in Chapter 12. Depending on the parameter values or on the reservoir concentration x_{0i} , there may be up to three steady states.

In Figure 2, the number of steady states is shown as a function of x_{0i} , for fixed values of the parameters σ , θ_x , and n . The corresponding generalized logical function is given by:

$$X_i = d_{x_i} (x_{0i} + \sigma x_i)$$

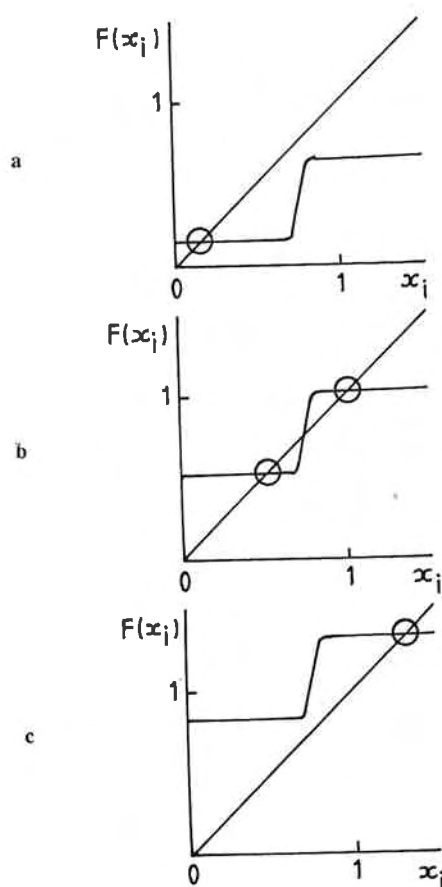


FIGURE 2. Steady states corresponding to Equation 1, for three values of the reservoir concentration, x_{0i} . Here, $\sigma = 0.5$, $\theta_x = 0.75$, and $n = 50$. (a) $x_{0i} = 0.150$; (b) $x_{0i} = 0.525$; (c) $x_{0i} = 0.825$. The stable steady states are circled.

with the state table:

x_i	X_i
0	K_0
1	K_1

where $K_0 = d_{x_i}(x_{0i})$ and $K_1 = d_{x_i}(x_{0i} + \sigma)$.

The specific situations corresponding to Figure 2 are shown in Table 1.

From Figure 2 and Table 1, we observe that even if the weight of the autocatalytic term is low ($\sigma < \theta_x$), the presence of a source may render the positive loop effective.

In the presence of diffusional coupling between the compartments, the composition in each cell will influence that in the other.

The equations for the time evolution of the concentrations in each compartment will contain an additional contribution describing the diffusion across the membrane, which is linearly proportional to the concentration difference:

$$\left(\frac{dx_1}{dt}\right)_{\text{diff.}} = \frac{D_x \cdot S}{e \cdot V_c} (x_2 - x_1)$$

TABLE 1

$$K_0 = K_1 = 0$$

x_i	X_i
0	0
1	0

$$x_{0i} + \sigma < \theta_x$$

$$K_0 = 0, K_1 = 1$$

x_i	X_i
0	0
1	1

$$x_{0i} < \theta_x < x_{0i} + \sigma$$

$$K_0 = K_1 = 1$$

x_i	X_i
0	1
1	1

$$\theta_x < x_{0i}$$

where D_x is the diffusion coefficient of product x across the membrane and e and S are the thickness and surface area of the membrane, respectively. The corresponding expression for $\left(\frac{dx_2}{d\Gamma}\right)_{\text{diff}}$ is obtained by permutation of the subscripts 1 and 2. Thus, for each compartment, we now have

$$\frac{dx_i}{d\Gamma} = \left(\frac{dx_i}{d\Gamma}\right)_{\text{flow}} + \left(\frac{dx_i}{d\Gamma}\right)_{\text{reaction}} + \left(\frac{dx_i}{d\Gamma}\right)_{\text{diff}}$$

or, more explicitly, the concentration of x within the two cells is governed by the system of coupled differential equations:

$$\begin{aligned} \frac{dx_1}{dt} &= x_{01} - x_1 + \lambda(x_2 - x_1) + \sigma \frac{x_1^n}{\theta_x^n + x_1^n} \\ \frac{dx_2}{dt} &= x_{02} - x_2 + \lambda(x_1 - x_2) + \sigma \frac{x_2^n}{\theta_x^n + x_2^n} \end{aligned} \quad (2)$$

where, as above, $t = T \cdot \frac{D}{V_c}$, $\sigma = k \cdot \frac{V_c}{D}$, and we have set $\lambda = \frac{D_x \cdot S}{e \cdot D}$. The parameter λ , called the mass transfer coefficient, is a measure of the diffusional coupling between the two cells. For simplicity, we have considered that the two compartments are identical, i.e., that each cell has the same characteristic time $\tau = V_c/D$ and that the reaction proceeds at the same rate (k) and with the same threshold value (θ_x) in the two cells.

In this chapter, we shall be interested in the steady-state solutions of System 2, and we will use the generalized logical description to study the stable states as a function of the reservoir concentrations (x_{0i}) and the mass transfer coefficient (λ).

III. A TWO-CELL SYSTEM: GENERALIZED LOGICAL DESCRIPTION

We saw in Chapter 8 that as $n \rightarrow \infty$, the sigmoid functions approach step functions and can be replaced by Boolean variables. The generalized logical functions corresponding to the piecewise linear equations are then readily constructed by discretization. In the present case, this is not possible in a straightforward way. To see this, let us write Equation(s) 2 in the form:

$$x_1 + \frac{1}{1 + \lambda} \cdot \frac{dx_1}{dt} = \frac{1}{1 + \lambda} \cdot (x_{01} + \sigma F^+(x_1) + \lambda x_2) = H_1(x_1, x_2) \quad (3.1)$$

$$x_2 + \frac{1}{1 + \lambda} \cdot \frac{dx_2}{dt} = \frac{1}{1 + \lambda} \cdot (x_{02} + \sigma F^+(x_2) + \lambda x_1) = H_2(x_1, x_2) \quad (3.2)$$

Each right-hand member, H_i ($i = 1, 2$), contains a linear term, $\frac{\lambda}{1 + \lambda} \cdot x_2$ and $\frac{\lambda}{1 + \lambda} \cdot x_1$, which can no longer be approximated by a step function. To derive the corresponding logical relations, one must first transform System 3 in such a way that these linear terms disappear. In the simple case considered here, this can be done in the following way. Into Equation 3.1, we insert the expression for x_2 from Equation 3.2, and into Equation 3.2, we insert the expression for x_1 from Equation 3.1. After collecting the terms, this leads to the system of equations*:

$$(1 + M_1)x_1 + \frac{dx_1}{dt} + M_1 \frac{dx_2}{dt} = \sigma F^+(x_1) + M_1 \sigma F^+(x_2) + x_{01} + M_1 x_{02}$$

$$(1 + M_1)x_2 + M_1 \frac{dx_1}{dt} + \frac{dx_2}{dt} = M_1 \sigma F^+(x_1) + \sigma F^+(x_2) + M_1 x_{01} + x_{02}$$

where $M_1 = \frac{\lambda}{1 + \lambda}$.

The steady-state solutions of System 2 or 3, which are defined by $\frac{dx_1}{dt} = \frac{dx_2}{dt} = 0$, are thus also given by the solutions of the algebraic equations:

$$\begin{aligned} (1 + M_1)x_1 &= \sigma F^+(x_1) + M_1 \sigma F^+(x_2) + x_{01} + M_1 x_{02} \\ (1 + M_1)x_2 &= M_1 \sigma F^+(x_1) + \sigma F^+(x_2) + M_1 x_{01} + x_{02} \end{aligned} \quad (4)$$

which now contain only sigmoid functions in their right-hand members. Introducing the variable change,

$$X_i = (1 + M_1)x_i, \quad i = 1, 2$$

the sigmoids become:

$$F^+(x_i) = \frac{x_i^n}{\theta_x^n + x_i^n} = \frac{X_i^n}{[(1 + M_1)\theta_x]^n + X_i^n} = \frac{X_i^n}{\theta^n + X_i^n} = F^+(X_i)$$

with the new threshold value:

$$\theta = (1 + M_1)\theta_x$$

In terms of the new variables, the algebraic System 4 may thus be written:

$$\begin{aligned} X_1 &= \sigma F^+(X_1) + M_1 \sigma F^+(X_2) + (x_{01} + M_1 x_{02}) \\ X_2 &= M_1 \sigma F^+(X_1) + \sigma F^+(X_2) + (M_1 x_{01} + x_{02}) \end{aligned} \quad (5)$$

* In mathematical terms, this amounts to inverting the transport matrix:

$$\begin{pmatrix} -(1 + \lambda) & \lambda \\ \lambda & -(1 + \lambda) \end{pmatrix}$$

It is worth pointing out that the coefficients that appear in these equations are related to one another by the following inequalities. Since λ , σ , and x_{0i} are each nonnegative, $M_1 = \frac{\lambda}{1 + \lambda} < 1$ and thus:

$$M_1\sigma < \sigma, \quad M_1x_{0i} < x_{0i} \quad (i = 1, 2)$$

Moreover,

$$\text{as } \lambda \rightarrow 0: M_1 \rightarrow 0 \text{ and } M_1\sigma \rightarrow 0, M_1x_{0i} \rightarrow 0$$

$$\text{as } \lambda \rightarrow \infty: M_1 \rightarrow 1 \text{ and } M_1\sigma \rightarrow \sigma, M_1x_{0i} \rightarrow x_{0i}.$$

The physical meaning of Equation(s) 5 is clear: when the cells communicate with one another by diffusion across their common boundary, the reaction and entry flux in compartment II will affect the steady-state concentration in compartment I, but in general to a lesser degree than the reaction and entry flux in compartment I itself, and vice versa.

We can now construct the logical relations corresponding to Equation(s) 5 that will determine the stable states of the two-cell system. These logical relations are*

$$\begin{aligned} X_1 &= d_x(\sigma x_1 + M_1\sigma x_2 + x_{01} + M_1x_{02}) \\ X_2 &= d_x(M_1\sigma x_1 + \sigma x_2 + M_1x_{01} + x_{02}) \end{aligned} \quad (6)$$

where we have taken into account that the discretization scales are the same for x_1 and x_2 since the thresholds are identical in the two compartments, i.e., $d_{x_1} = d_{x_2} = d_x$.

Let us first consider the case in which the concentrations in the two reservoirs are equal ($x_{01} = x_{02} = x_0$). The two cells are thus completely identical. We set:

$$x_{01} + M_1x_{02} = M_1x_{01} + x_{02} = (1 + M_1)x_0 = K_0$$

The corresponding general state table is given in Table 2.

TABLE 2

x_1	x_2	X_1	X_2
0	0	K_0	K_0
0	1	K_1	K_2
1	1	K_3	K_3
1	0	K_2	K_1

with the logical parameters $K_0 = d_x(K_0)$, $K_1 = d_x(K_0 + M_1\sigma)$, $K_2 = d_x(K_0 + \sigma)$, and $K_3 = d_x[K_0 + (1 + M_1)\sigma]$. The particularity here is that, for values of $\lambda \neq 0$ or ∞ , $0 < M_1 < 1$, and

* More precisely, the discrete application is constructed starting from a piecewise linear differential system whose right-hand member is equivalent to Equation(s) 5. The steady states of the two systems are identical, but the dynamics will, in general, be different. The information that one can gain from the logical method as applied here is thus, in general, limited to the steady-state behavior.

all these logical parameters are related to one another through the inequalities:

$$K_0 < K_0 + M_1\sigma < K_0 + \sigma < K_0 + (1 + M_1)\sigma. \quad (7)$$

which must be taken carefully into account when ascribing logical values to the different parameters.

IV. STABLE STATES AS A FUNCTION OF THE RESERVOIR CONCENTRATION AND THE STRENGTH OF COUPLING

Let us investigate the steady-state behavior when the reservoir concentration x_0 or K_0 varies, λ and σ being held constant so that $0 < M_1 < 1$ and $(1 + M_1)\sigma < \theta$.

We start with extremely low values of K_0 such that:

$$K_0 + (1 + M_1)\sigma < \theta$$

In terms of the logical parameters, this means that:

$$K_3 = K_2 = K_1 = K_0 = 0$$

As the source strength is increased, the logical parameters will successively switch from the logical value 0 to 1, following the order determined by the inequalities (7), until K_0 itself reaches the threshold value θ , making $K_0 = 1$.

Figure 3.1 shows the successive state tables for increasing values of K_0 . For each case, the corresponding steady-state curves (5) and the nullclines of the initial differential System 2 are also plotted.

From the state tables, it can be seen that as the reservoir concentration increases, multiple stable states appear. There are two ranges of external concentrations for which two stable symmetric states coexist, corresponding to low $\textcircled{00}$ and high $\textcircled{11}$ concentrations of x in both compartments. These two bistability regions are separated by a region with four stable states, including two new asymmetric states, $\textcircled{01}$ and $\textcircled{10}$, with unequal concentrations in the two cells. These asymmetric concentration patterns are stable despite the fact that the cells are otherwise identical. For low or high source strengths, there is a unique stable state, $\textcircled{00}$ or $\textcircled{11}$, respectively. The fit with the continuous description is illustrated in Figures 3.2 and 3.3, which show, in addition, the distortion of the nullclines by the transformations described in Section III.

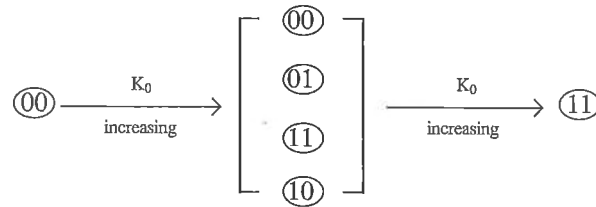
Let us now investigate how the multiplicity of stable states varies as a function of the mass transfer coefficient λ , which measures the intensity of diffusional coupling between the two cells.

In the limit $\lambda = 0$ or $M_1 = 0$ (no coupling), we have

$$K_1 = K_0 \text{ and } K_3 = K_2$$

Inserting this in the general state table (Table 2), it is clear that for increasing values of the

external concentration, there will be successively one, four, and then again one stable state:

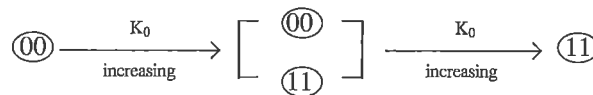


In each case, the stable situations simply correspond to the combination of states that are available to each cell since the compartments are totally independent.

In the limit $\lambda = \infty$ or $M_1 = 1$, on the other hand, we have

$$K_1 = K_2$$

and the compartmentalized system behaves as a single cell (see Section II).



In Figure 4 is summarized the steady-state behavior of the two-cell system as a function of both x_0 and λ for fixed values of σ and θ_x . This diagram is obtained from Table 2 by evaluating the logical parameters for different values of x_0 and λ . The limits (a), (b), (c), and (d) of the different domains are determined by:

$$(a): K_0 + (1 + M_1)\sigma = \theta$$

$$(b): K_0 + \sigma = \theta \text{ (with } K_0 + M_1\sigma < \theta)$$

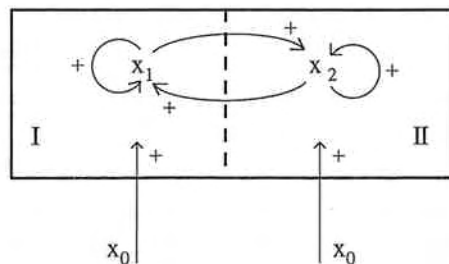
$$(c): K_0 + M_1\sigma = \theta$$

$$(d): K_0 = \theta$$

with $K_0 = (1 + M_1) x_0$ and $\theta = (1 + M_1) \theta_x$.

For low values of coupling between the two cells, there is a region in which four stable states coexist. When the coupling strength increases, this region becomes narrower and narrower and finally disappears at the limit of infinite coupling ($M_1 = 1$).

As can be seen from Equations (5) or from the logical equations (6), the coupled cells can be represented by the graph of interactions:



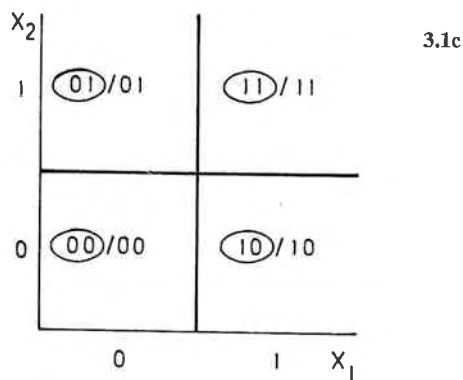
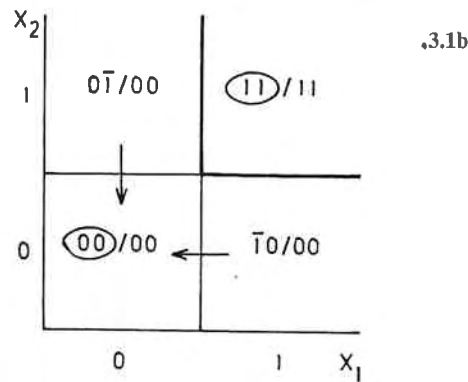
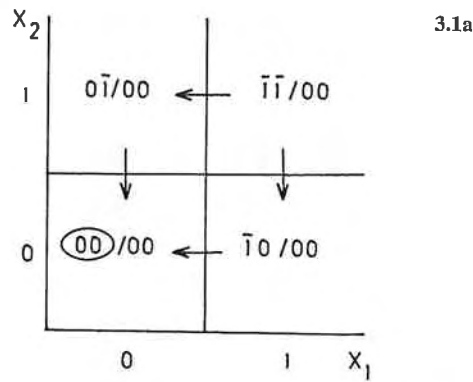


FIGURE 3.1 Stable states for increasing values of the reservoir concentration x_0 or $K_0 = (1 + M_1)x_0$. (3.1) State tables corresponding to the generalized logical equation(s) 6. (a) $K_3 = K_2 = K_1 = K_0 = 0$; (b) $K_3 = 1, K_2 = K_1 = K_0 = 0$; (c) $K_3 = K_2 = 1, K_1 = K_0 = 0$; (d) $K_3 = K_2 = K_1 = 1, K_0 = 0$; (e) $K_3 = K_2 = K_1 = K_0 = 1$.

On the one hand, for each compartment, the existence of a source term of a certain intensity is necessary in order to observe multistationarity when $\sigma < \theta_x$. On the other hand, the diffusional coupling between the cells or positive loops tends to decrease the number of stable states as a function of the strength of coupling. Although passive diffusion creates an additional positive loop, as long as the external and internal parameters remain the same for

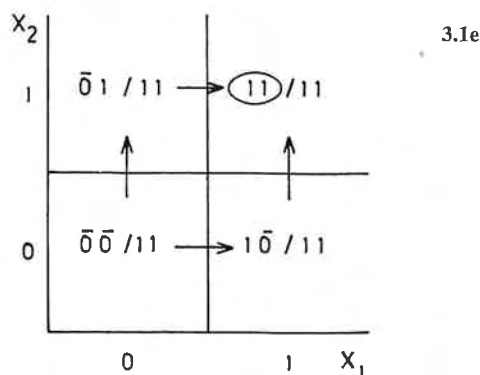
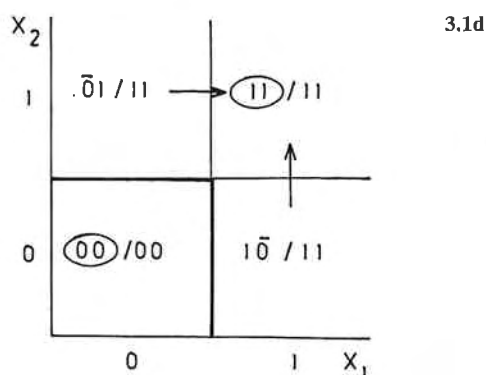


FIGURE 3.1. Continued.

the two compartments, this will not generate additional stable states because of the restrictive conditions on the logical parameter values. In particular, the stable asymmetric states (01) and (10) will always coexist and a situation with three stable states is impossible here.

V. HOW TO REACH A STABLE ASYMMETRIC CONCENTRATION PATTERN

To reach one of the stable asymmetric states, the system being otherwise perfectly symmetric, one can modify the internal or external conditions.

In the first case, both cells are maintained at the same constant external concentration x_0 . The choice of the *initial* concentrations inside each compartment will determine the final state of the system, according to whether the initial state is on one or the other side of the separatrices (see Figure 3.2).

In the second case, the initial conditions are such that the concentrations inside the two compartments and the external concentrations are symmetric, i.e., $x_1 = x_2$, $x_{01} = x_{02}$. The asymmetric stable states can then be reached by acting *temporarily* on the concentrations in one of the reservoirs (x_{01} or x_{02}), all other parameters being held constant.

Let us consider an example. We start with equal reservoir concentrations, $x_{01} = x_{02} = x_0$, corresponding to the multistability region with four stable states (Table 4a) and choose initial concentrations within the cells corresponding to the stable upper symmetric state (11) . We then apply a constant perturbation to reservoir 1: x_{01} is decreased by a quantity μ , while

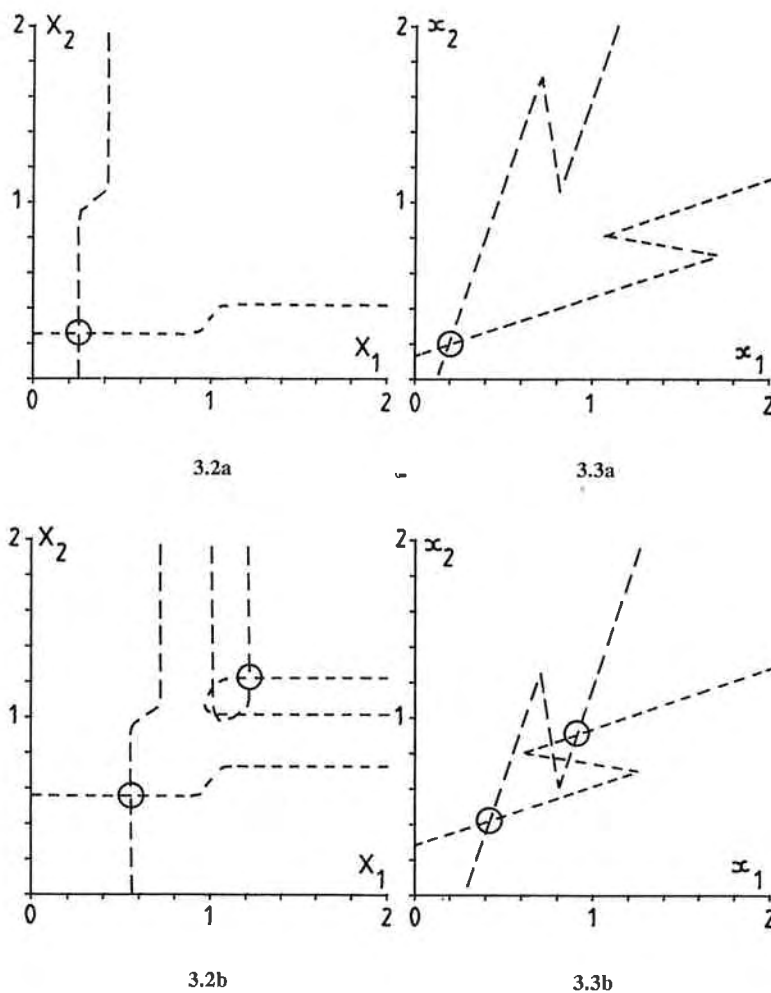


FIGURE 3.2. Steady-state curves corresponding to System 5. The intersects of the curves give both the stable and unstable steady states. The stable steady states are circled. The parameter values are in agreement with the logical description: $\sigma = \lambda = 0.5$, $\theta_x = 0.75$, and $n = 50$. (a) $K_0 = 0.25$; (b) $K_0 = 0.55$; (c) $K_0 = 0.65$; (d) $K_0 = 0.80$; (e) $K_0 = 1.1$.

FIGURE 3.3. Nullclines corresponding to System 2. $\frac{dx_1}{dt} = 0$, long dashes (—); $\frac{dx_2}{dt} = 0$, short dashes (- -). The parameter values are those corresponding to Figure 3.2. Here again, the stable steady states are circled.

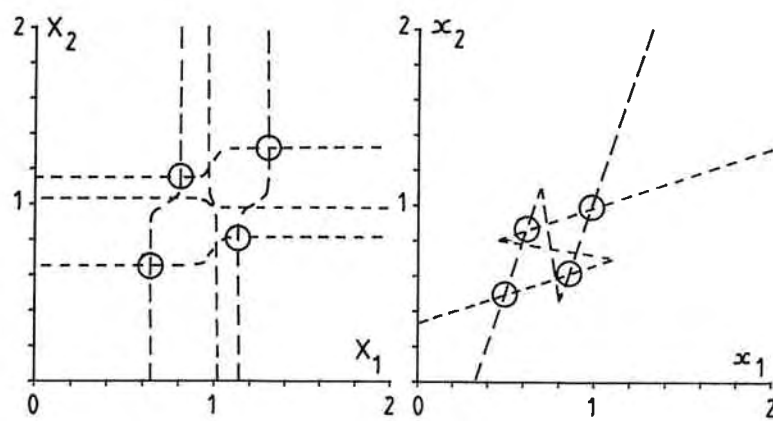
x_{02} and all the other parameters remain unchanged. The source terms in the generalized logical equations (6) thus become:

$$x_{01} + M_1 x_{02} = (1 + M_1) x_0 - \mu = K_0 - \mu = K_{01}$$

and

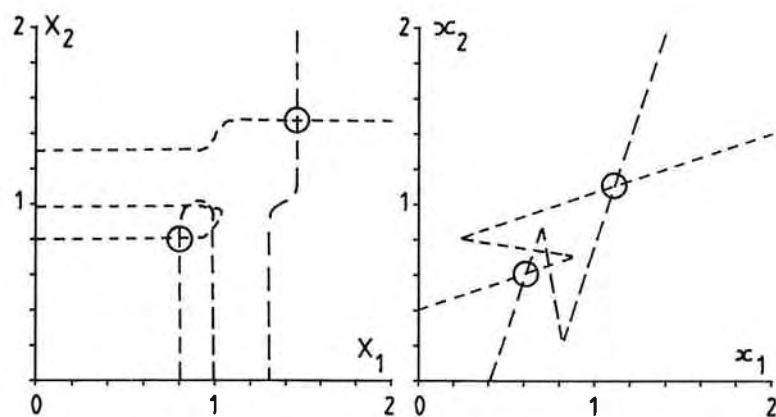
$$M_1 x_{01} + x_{02} = (1 + M_1) x_0 - M_1 \mu = K_0 - M_1 \mu = K_{02}$$

with $K_{01} < K_{02}$. The corresponding general state table is now given by:



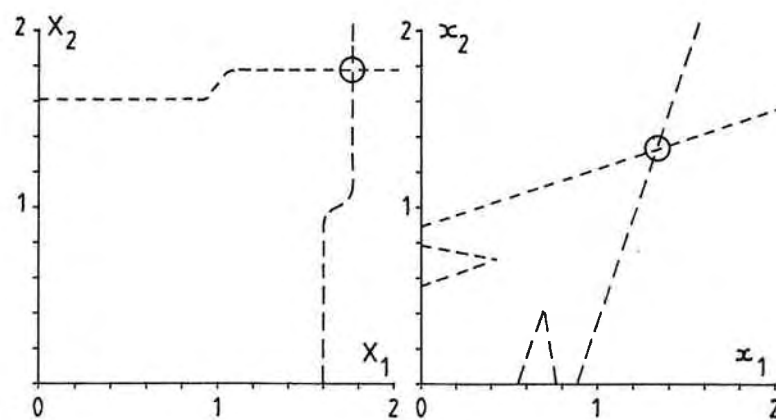
3.2c

3.3c



3.2d

3.3d



3.2e

3.3e

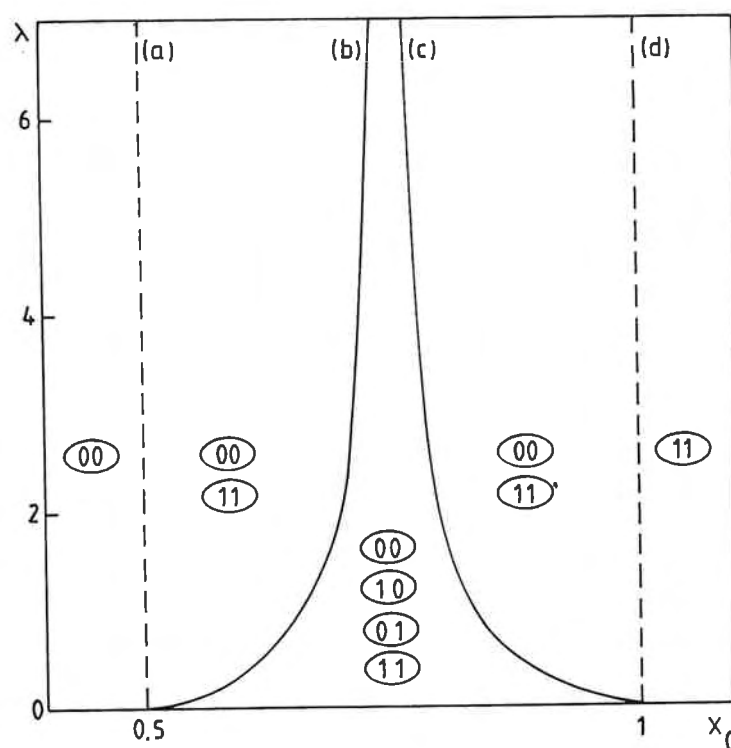


FIGURE 4. Domains of existence of the different stable states as a function of the reservoir concentration x_0 and the mass transfer coefficient λ . Here, $\sigma = 0.5$ and $\theta_x = 1$.

TABLE 3
General State Table for Unequal Source Terms: $x_{01} = x_{02} - \mu$

x_1	x_2	X_1	X_2
0	0	$d_x(K_{01})$	$d_x(K_{02})$
0	1	$d_x(K_{01} + M_1\sigma)$	$d_x(K_{02} + \sigma)$
1	1	$d_x[K_{01} + (1 + M_1)\sigma]$	$d_x[K_{02} + (1 + M_1)\sigma]$
1	0	$d_x(K_{01} + \sigma)$	$d_x(K_{02} + M_1\sigma)$

In each column, the logical parameters are related to one another by inequalities (7), in which K_0 is replaced by K_{01} or K_{02} . It is easy to show that, provided the concentration difference μ satisfies the conditions

$$\mu > \lambda\sigma \text{ and } K_0 + (1 + M_1)\sigma - \theta < \mu \leq \frac{1}{M_1} (K_0 + \sigma - \theta)$$

these new logical parameters will take values that lead to Table 4b.

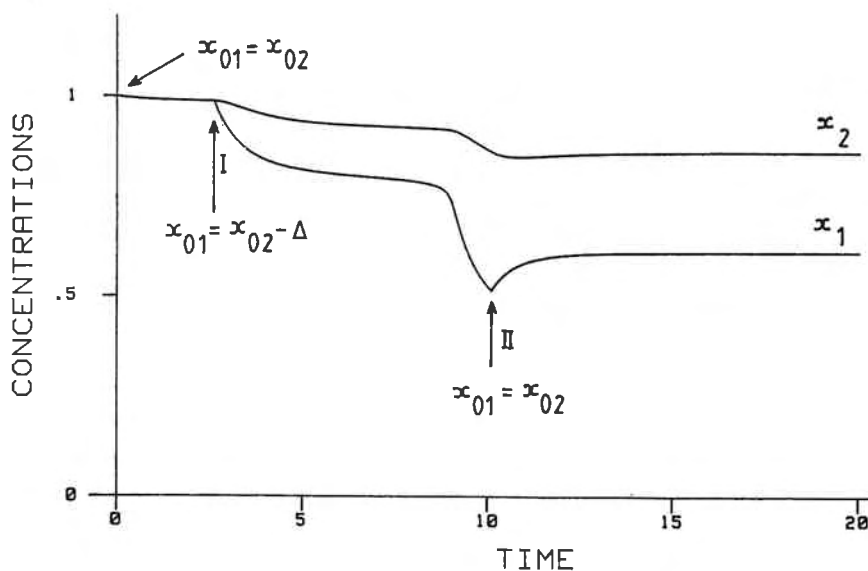


FIGURE 5. Time evolution of the concentrations x_1 and x_2 obtained by numerical integration of Equation(s) 2 with the parameter values: $\sigma = \lambda = 0.5$, $\theta_x = 0.75$, and $n = 50$. The initial concentrations within the cells and boundary conditions are $x_1 = x_2 = 1$, $x_{01} = x_{02} = 0.4875$. At time $t = 5$ (arrow I), an external perturbation, $\Delta = -0.24$, is applied in reservoir 1, x_{02} being held constant. At time $t = 10$ (arrow II), the symmetry of the reservoir concentrations is restored ($\Delta = 0$). Under these conditions, the system reaches an asymmetric steady state.

TABLE 4
Compact State Tables

$x_1 x_2$		$x_1 x_2$		$x_1 x_2$
⓪⓪		⓪⓪		⓪⓪
⓪1		⓪1	$\xrightarrow{\mu=0}$	⓪1
11	$\xrightarrow{\mu}$	1̄1		11
10		1̄0		10
(a)		(b)		(c)
$x_{01} = x_{02} = x_0$		$x_{01} = x_{02} - \mu$		$x_{01} = x_{02} = x_0$

Now, state 11 is no longer a stable state and the system will evolve toward the stable asymmetric state ①1. When the perturbation, μ , is removed and the symmetry of the external conditions is restored (Table 4c), the system will remain in the stable asymmetric state ①1. Here, tables 4a and 4c are derived from (the general state) Table 2, and Table 4b corresponds to (the general state) Table 3. The asymmetric stable state ①0 can be reached in a similar way, by transiently decreasing the concentration x_{02} in reservoir 2.

The correspondence with the differential description (2) is illustrated in Figure 5, which shows the time evolution of the concentration of x in compartments I and II. Prior to the perturbation, the concentrations x_1 and x_2 are always equal. When an external perturbation Δ is applied, the concentration pattern becomes asymmetric. This asymmetry persists when the perturbation is removed ($\Delta = 0$).

VI. CONCLUSION

In this chapter, we have applied the generalized logical method to the study of a compartmentalized system. The coupling of neighboring cells by passive diffusion of the reactants creates conditions on the logical parameters, and the determination of the stable states amounts simply to evaluating a set of inequalities.

Depending on the mass transfer coefficient (λ) and the reservoir concentrations (x_{0i}), the steady-state problem can have multiple solutions, including asymmetric stable states. The description that is given here is consistent with the continuous formalization and previous studies on compartmentalized systems.⁴⁻⁷

The logical method can easily be extended to the study of more than two compartments in various spatial configurations or with different reaction characteristics inside the cells.⁸

This example illustrates how the interplay between reaction kinetics and diffusion processes can generate stable differentiated states (in the form of spatially structured concentration patterns), although the internal and external constraints remain perfectly symmetric.

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Chapter 19

THE SOS RESPONSE**TABLE OF CONTENTS**

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I. INTRODUCTION

Living organisms lavish exquisite care on their genetic material, DNA. The bacterium *Escherichia coli*, for example, is able to duplicate its chromosome of 5 million base pairs with an overall accuracy of 10^{-10} errors per base pair. In addition, it possesses a number of DNA repair enzymes capable of removing or repairing a wide variety of chemical lesions that can occur in the DNA. Some of these enzymes are inducible by DNA-damaging treatments as a part of what has come to be known as the "SOS response".¹ The regulation of the SOS response posed an interesting genetic and biochemical problem. Although the SOS response seemed philosophically similar to the lactose operon — specific enzymes are synthesized only when they are needed — the nature of the inducer was far from obvious: induction was observed after a large number of chemical and physical treatments having little in common (e.g., treatment with mitomycin C, nalidixic acid, ultraviolet or X irradiation). The combined efforts of a number of laboratories ultimately succeeded in unraveling the regulatory scheme of the SOS response, which we present here.

II. REGULATION OF THE SOS RESPONSE

The SOS functions are repressed by a transcriptional repressor, coded for by the *lexA* gene. When DNA is damaged, the replication fork may stall at the site of the lesions. This results in local degradation of the newly synthesized strand, creating a stretch of single-stranded DNA. The RecA protein binds to the single-stranded DNA and, in the presence of ATP (or deoxyATP), forms a ternary complex with a remarkable property: it catalyzes the proteolytic cleavage of the LexA repressor at a specific site, inactivating it and derepressing the SOS functions. The degradation that creates the single-stranded DNA need not be at the replication fork. Certain DNA lesions are recognized by constitutive enzymes, leading to degradation near the lesions, even if there is no replication fork present.

To complete the picture, we must add that the regulatory proteins, LexA and RecA, are themselves SOS functions in the sense that their expression is repressed by LexA. In both cases, however, the repression is only partial, and, indeed, the residual expression is of a fundamental physiological importance: the basal level of LexA ensures the repression of the other SOS functions in the absence of DNA damage, and the basal level of RecA ensures the inducibility of the SOS response when needed. The autorepression of LexA results in a homeostatic control, tending to maintain a constant concentration of the LexA repressor.

The overall picture can be summarized simply: DNA damage results in localized degradation, producing single-stranded stretches of DNA. The RecA protein, in the presence of single-stranded DNA, cleaves the LexA repressor.

It is interesting that under certain conditions, the RecA protein also cleaves the repressors of certain temperate bacteriophages, the best known of which is λ (cf. Chapter 20). The study of SOS response thus established the molecular bases of the phenomenon of lysogenic induction.

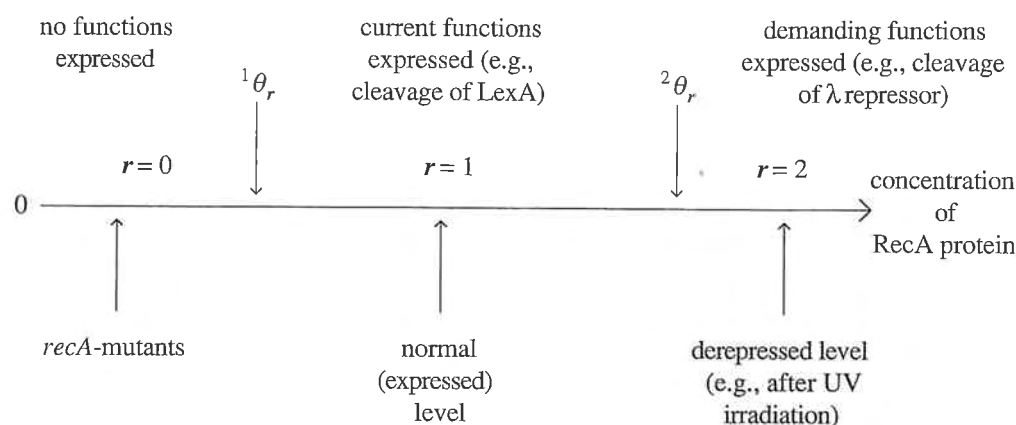
III. FORMALIZATION OF THE SOS RESPONSE

A. PRELIMINARY CONSIDERATIONS

Our description will obviously include the level of RecA (variable r , function R), and of LexA (I and L). To account for induction, we also include a variable s that takes the value 1 if there is single-stranded DNA present in the cell, 0 otherwise.

We know that there are essentially three steady levels of RecA protein: derepressed in the absence of LexA protein, repressed (by the *lexA* product) in normal conditions, and nil in

recA⁻ mutants. We might be tempted to build the discretized scale of a variable r on the basis of these three levels. However, our policy has been to discretize the scale of a variable not on the basis of its steady levels, but according to the number of interactions it has with other elements, i.e., the number of levels required to obtain different effects. Since we know that some functions of protein RecA are efficiently expressed at moderate concentrations (e.g., the cleavage of LexA protein), whereas others (like the cleavage of λ repressor) require significantly higher concentrations, we will consider two thresholds: ${}^2\theta_r$, below which the latter functions are not expressed, and ${}^1\theta_r$, below which none of the RecA functions are expressed. The diagram



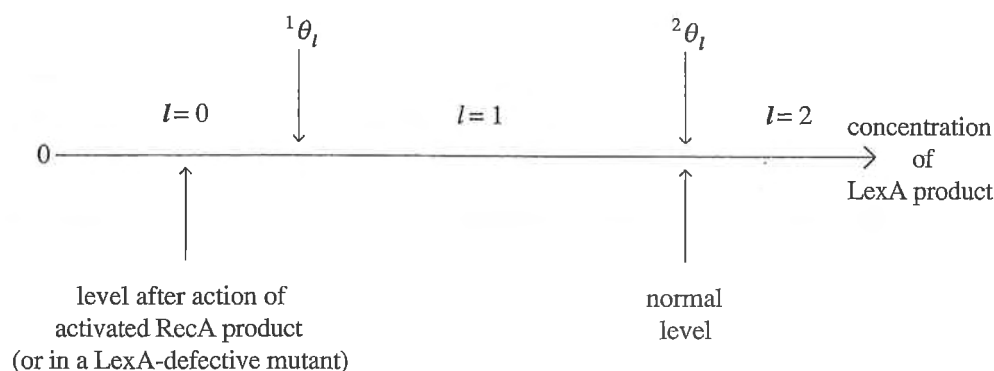
illustrates the fact that the three steady levels considered above *happen* to correspond to the logical levels 2, 1, and 0, respectively, defined on the basis of the effects of the RecA protein on its targets. As usual, we associate with the three-valued variable r the two binary variables 1r and 2r , which are 0 for $r < 1$ and $r < 2$, respectively, and 1 otherwise.

As discussed in the previous section, the LexA protein exerts a negative control on its own expression. We know from other examples that the differential description of this situation has a steady level near the autoregulation threshold θ_1 , whereas in the naïve logical description, l oscillates. As mentioned in Chapter 8, this type of steady state can be identified in the logical description as well by introducing the threshold value θ in the state table. Thus, in a preliminary description of LexA autoregulation (not accounting for induction), we would set $L = \bar{l}$ and write the state table

l	L
0	1
θ	θ
1	0

from which it is clear that the steady state level is indeed θ . However, under normal conditions, the *lexA* product exerts *efficient* negative control on various other genes. This implies that the normal steady level of LexA protein is higher than its threshold concentration(s) for these controls. Thus, we must associate with the *lexA* product a threshold ${}^2\theta_1$ for its autoregulatory function and a threshold ${}^1\theta_1$ for its other functions. A detailed description involving the expression of several specific SOS functions might require several thresholds less than the autoregulation threshold. For our purposes, however, we will assume that

induction occurs at the same threshold for all functions. The variable (I) and function (L) associated with the *lexA* product will thus have three logical values. The diagram



shows the correspondence between the two steady levels (below) and the discretized scale (above). The binary variables 1I and 2I equal 0 for $l < 1$ and $l < 2$, respectively, and 1 otherwise. In view of the importance of the level $^2\theta$ in the present case, we will include it explicitly in the state tables, thus considering the values 0, 1, $^2\theta$, and 2 for I and L .

B. FORMALIZATION

As already mentioned, the *recA* gene is under negative control of LexA, but is expressed at a significant basal level, even in the presence of the LexA protein. This can be written:

$$R = d_r(K_0 + K_1 \bar{I})$$

Since the basal level is sufficient for those RecA functions that define logical level 1, whereas only the derepressed level permits the expression of the RecA functions that define logical level 2, we take:

$$d_r(K_0) = K_0 = 1$$

$$d_r(K_0 + K_1) = K_{0+1} = 2$$

If, in addition, we wish to take into account the possible use of *recA*⁻ mutants in the experiments, we use the input variable g_r and write:

$$R = g_r \cdot d_r(K_0 + K_1 \bar{I})$$

in which $g_r = 1$ if the *recA* gene is normal, $g_r = 0$ if it is mutationally inactivated. The state table is thus:

R	0	1	g_r
0	0	K_{0+1}	
1	0	K_0	
1I			

For $K_0 = 1$ and $K_{0+1} = 2$, we have

R	0	1	g_r
0	0	2	
1	0	1	

I

For the moment, we will reason in terms of $recA^+$ bacteria ($g_r = 1$), and only after describing their behavior will we introduce additional input variables describing genetic abnormalities.

To formalize the LexA function, we must describe the following situation (see Section I): (1) the *lexA* gene is under negative control of the LexA protein, with threshold $2\theta_l$. This will involve a term $\bar{2}l$ in the expression for L . (2) Product LexA is destroyed if the RecA product is present at a level > 1 (which is the case unless the cell has a $recA^-$ mutation) AND there are single-stranded regions ($s = 1$) in the DNA as a result of damage (UV irradiation, etc.). Thus, a condition to have $L \neq 0$ is $\bar{1}rs$ or $\bar{1}\bar{r} + \bar{s}$. Combining (1) and (2), we have

$$L = d_1[K_2 \cdot \bar{2}l \cdot (\bar{1}\bar{r} + \bar{s})]$$

As for single-stranded DNA (variable s), we can reason that once induced by an appropriate agent, it will persist unless the LexA protein disappears, thus permitting the expression of the SOS repair functions. This can be symbolized:

$$S = (\phi + s) \cdot I$$

in which ϕ represents the various agents capable of inducing the lesions. (The exact nature of these agents does not interest us here.) In this schematic description, the DNA repair functions under LexA control are not explicitly formalized. The time required for their synthesis and effective action once LexA is cleaved can be assimilated to the off delay relating S to I . The overall picture is thus:

$$L = d_1[K_2 \cdot \bar{2}l \cdot (\bar{1}\bar{r} + \bar{s})]$$

$$R = d_r(K_0 + K_1 \bar{1}l) \quad (1)$$

$$S = (\phi + s) \cdot I$$

As we have seen, it is advisable to take $K_0 = d_r(K_0) = 1$ and $K_{0+1} = d_r(K_0 + K_1) = 2$. This is not actually necessary at this point, but will be below when we include the induction of λ , which requires more than the basal level of RecA protein. As for K_2 , the retroaction of L on itself will have a steady state $2\theta_l$ only if $K_2 > 1$. We therefore take $K_2 = 2$.

As the appearance of single-stranded DNA can be induced at will by appropriate injuries, it is convenient to use a column for the normal ($s = 0$) situation and a column ($s = 1$) for the presence of single-stranded DNA as though s were an input variable. (Of course, it is not: s is an internal variable with an associated function S that depends on the other variables of the system.) As a result of an appropriate injury, $s = 1$ and the system shifts from the left to the right column. However, since in the presence of the RecA protein, single-stranded DNA results in cleavage of the LexA protein, the SOS genes are derepressed and DNA is soon repaired. Then we again have $s = 0$ (shift back to the left column).

The state tables are shown in Table 1.

TABLE 1
The Complete (a) and Compact (b) State Tables of System 1

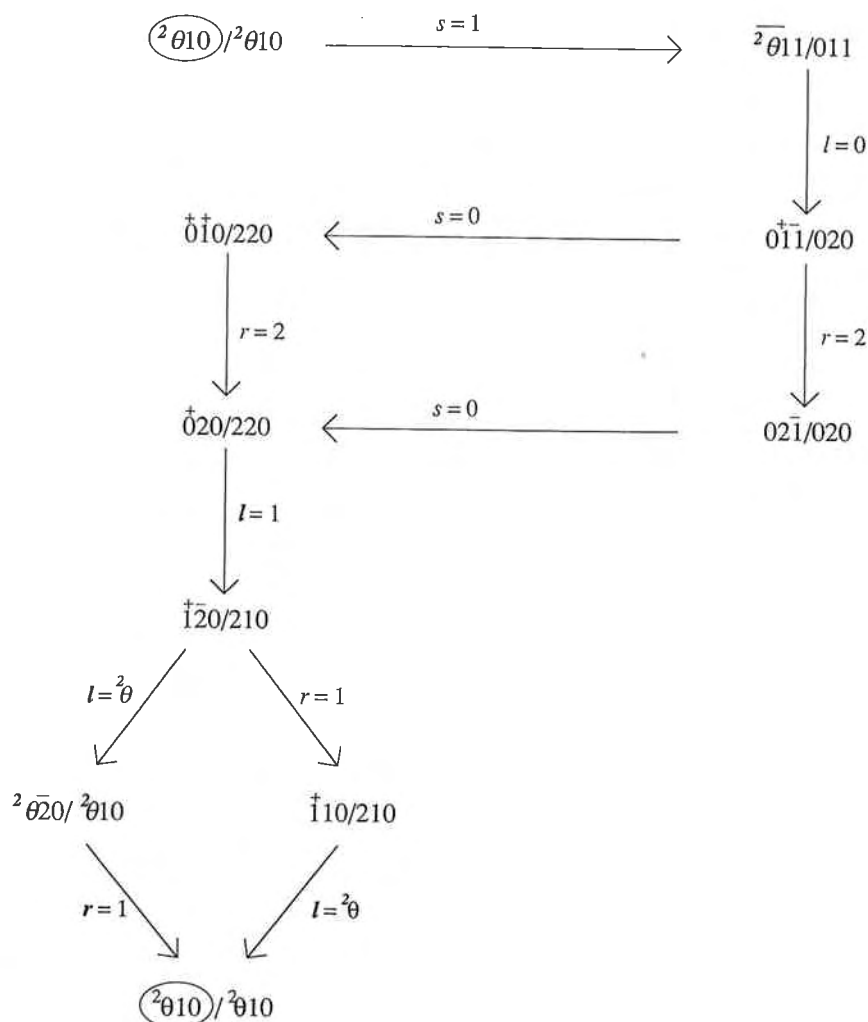
(a)

L, R, S	0	1	s
0 0	220	220	
0 1	220	020	
0 2	220	020	
1 0	210	211	
1 1	210	011	
1 2	210	011	
${}^2\theta$ 1	${}^2\theta 10$	011	
2 0	010	011	
2 1	010	011	
2 2	010	011	
l, r			

(b)

0	1	s
$\bar{0}\bar{0}\bar{0}/220$	$\bar{0}\bar{0}\bar{1}/220$	
$\bar{0}\bar{1}\bar{0}/220$	$\bar{0}\bar{1}\bar{1}/020$	
$\bar{0}\bar{2}\bar{0}/220$	$\bar{0}\bar{2}\bar{1}/020$	
$\bar{1}\bar{0}\bar{0}/210$	$\bar{1}\bar{0}\bar{1}/211$	
$\bar{1}\bar{1}\bar{0}/210$	$\bar{1}\bar{1}\bar{1}/011$	
$\bar{1}\bar{2}\bar{0}/210$	$\bar{1}\bar{2}\bar{1}/011$	
$\textcircled{{}^2\theta 10} / {}^2\theta 10$	${}^2\theta 11/011$	
$\bar{2}\bar{0}\bar{0}/010$	$\bar{2}\bar{0}\bar{1}/011$	
$\bar{2}\bar{1}\bar{0}/010$	$\bar{2}\bar{1}\bar{1}/011$	
$\bar{2}\bar{2}\bar{0}/010$	$\bar{2}\bar{2}\bar{1}/011$	

We see that in the absence of single-stranded DNA (left column), there is a steady state $(^2\theta 10)/^2\theta 10$. If we introduce single-stranded DNA (shift to the right column):



Note that throughout this process, the level of RecA has remained ≥ 1 , that is, sufficient for the activities we are explicitly considering in this formalization. Strictly speaking, one could thus drop variable r and reason solely in terms of LexA and single-stranded DNA. However, if we are interested in λ induction (and we are, indeed), we must distinguish between $r = 2$ (which induces λ) and $r = 1$ (which does not). If we let I represent the induction of λ , we have $I = ^2rs$. In actual fact, the amplification of RecA protein that accompanies the induction of the SOS response is directly beneficial to the bacterium (if it is not lysogenic for λ). The RecA protein is also capable of catalyzing certain types of genetic recombination that make it possible to replicate DNA, even in the presence of lesions that are not a substrate for the DNA polymerase. This activity of RecA is a typical SOS function, inducible and involved in DNA repair. The protease activity, on the other hand, is regulatory and must be functional with the basal level of RecA protein.

Now, we will include the possible use of mutated strains in the analysis. To include *RecA*-defective mutations, we already introduced variable g_r (for gene *recA*); $g_r = 1$ in the wild type, 0 in a *recA* mutant.

Similarly, we introduced variable g_b , which takes the value 0 in a *lexA*-defective mutant.

There also exist mutations that render the *lexA* product noncleavable. In such a mutant, the SOS response cannot be induced, and the strain is therefore highly sensitive to treatments (like UV irradiation) that damage DNA and require repair functions for survival. To account for this allele, we introduce the variable ng_l (for LexA noninducible), which takes the value 1 if such a mutation is present, 0 otherwise. Since this type of mutation does not inactivate LexA (indeed, LexA continues to repress the SOS functions, even in the presence of RecA plus single-stranded DNA), one can have $g_l = 1$ and $^ng_l = 1$ simultaneously. The relationship for L becomes

$$L = g_l \cdot d_l [K_2 \cdot \overline{^2I} (\overline{^1R} + \overline{s} + ^ng_l)]$$

There also exists a *recA* mutation rendering its proteolytic activity constitutive (i.e., independent of the presence of single-stranded DNA created by DNA lesions). Such a mutant will have no LexA protein and, therefore, a high level of RecA and the other SOS functions unless its LexA protein happens to be noncleavable ($^ng_l = 1$). To account for this *recA* allele, we introduce the variable cg_r , which takes the value 1 if there is such a mutation, 0 otherwise. To see how this variable should be included in the logical relations, we reason as follows: LexA expression (L) has a genetic component (g_l), an autoregulatory component ($\overline{^2I}$), and an induction component. The last can be expressed as $\overline{\text{protease}} + ^ng_l$ (absence of protease OR presence of noncleavable LexA protein), and $\text{protease} = ^1r \cdot s + ^1r \cdot ^cg_r$, in which the first term represents "normal" induction (via DNA damage) and the second, "genetic" induction (via a protease-constitutive mutation).

The induction component of LexA expression is thus:

$$\overline{^1r(s + ^cg_r)} + ^ng_l = \overline{^1r} + \overline{s} + \overline{^cg_r} + ^ng_l = \overline{^1r} + \overline{s} \cdot \overline{^cg_r} + ^ng_l$$

The logical equations become:

$$L = g_l \cdot d_l [K_2 \cdot \overline{^2I} (\overline{^1r} + \overline{s} \cdot \overline{^cg_r} + ^ng_l)]$$

$$R = g_r d_r (K_0 + K_1 \overline{^1I})$$

$$S = (\phi + s) ^1I$$

In practice, it is convenient to treat separately each combination of values of the genetic input variables. For example, if $g_l = g_r = 1$ and $^ng_l = ^cg_r = 0$, we have the normal (wild-type) genetic situation, described by the relations in Equation 1. If $g_l = g_r = ^cg_r = 1$ and $^ng_l = 0$, the LexA protein is normal, but the RecA protein has constitutive protease activity, we have

$$L = d_l [K_2 \cdot \overline{^2I} \overline{^1r}]$$

$$R = d_r (K_0 + K_1 \overline{^1I})$$

$$S = (\phi + s) ^1I$$

Asssuming, as we have, that $d_r(K_0) \geq 1$, i.e., that the basal level of RecA is sufficient to cleave LexA efficiently, it is clear that $R \geq 1$ and, therefore, ultimately $\overline{r} = 0$. Therefore, in this system ($c_{gr} = 1$), ultimately we will have $L = 0$. There will thus be a high constitutive level of RecA and all other SOS functions. It is amusing to note, however, that if the induced level of RecA were required for LexA cleavage, i.e., $d_r(K_0) = 0$, but $d_r(K_0 + K_1) \geq 1$, we would have a positive loop with two stable states: either the basal level of RecA and threshold level of LexA ($r = 0, l = 2\theta_l$) or a high level of RecA and no LexA ($r = K_{\theta+1}, l = 0$).

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Chapter 20

THE BACTERIOPHAGE λ

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I. INTRODUCTION TO LYSOGENY

The study of bacteriophage λ was one of the most sophisticated fields in the young science of molecular biology. As Francis Crick once said, "The most fashionable disease among molecular biologists is a λ headache" (or words to that effect). Why this interest for a small bacteriophage among many others? One reason, no doubt, was the general admiration for the early work of Jacob. The discovery of the principles of biological regulation by Jacob and Monod was based on parallel work on the *Escherichia coli* lactose genes and on bacteriophage λ . The complementarity of these two lines of research played an essential role; in fact, realizing that the two systems have deep functional similarities was a stroke of genius.¹

Another reason for the success of bacteriophage λ is the fact that it is the prototype of temperate bacteriophages. As shown by Lwoff,² these viruses have a double life. On the one hand, they can multiply at the expense of their host cells and kill them, like other viruses. On the other hand, they can establish a symbiotic relation with their host cell, resulting in lysogenic bacteria in which phage and host have become a single unit.

This double life is of interest for at least two reasons. First, it is a well-defined biological system in which the same genetic material can behave in different ways under apparently identical environmental conditions, and of all such systems, it is one of the smallest (we almost wrote "simplest", but as we shall see, the system is anything but simple^{3,18,19}). In other words, the λ -host interaction is an excellent model system for the study of *differentiation*, in which the behavior is governed by epigenetic changes of the type described in Chapter 17. Second, as suggested long ago by Jacob⁴ and amply confirmed since, there are deep behavioral similarities between temperate bacteriophages and certain cancer viruses (a number of these properties had to be rediscovered in cancer virus-animal cell systems 20 years after the work on the λ -bacterium system).

When a typical DNA phage infects a cell, it injects its genetic material (a DNA molecule) into the cell, and the cellular protein-synthesizing and energy-producing machinery is used by the virus to replicate its own DNA, synthesize its own proteins, and, finally, lyse the bacterial cell, typically liberating hundreds of viral particles. Temperate phage like λ can behave this way, but, as already mentioned, they can also lysogenize the bacterial cell. In this case, the viral DNA molecule is integrated at a specific site in the bacterial chromosome, characteristic for each phage type; it is then called a prophage. As first hypothesized by Campbell,⁵ the phage DNA molecule, which is linear in the phage particle, circularizes as it enters the bacterial cell. Phage functions can then catalyze a crossing over between specific sites in the bacterial and viral chromosomes, thus converting a big and small circle into a bigger circle (Figure 1). On the other hand, the viral *cI* gene synthesizes a repressor that blocks the expression of other viral genes, thus rendering them harmless for the bacterium. How can the phage perpetuate itself while its genes, including those involved in replication, are blocked by the repressor? Simply because its DNA has become part of the bacterial chromosome. As such, it is replicated passively and faithfully transmitted to all the progeny. The viral DNA has become part of the genetic patrimony of the bacterium. The fact that a λ lysogen contains λ -specific repressor in its cytoplasm prevents the expression not only of the prophage genome it carries, but also of other λ phage that might infect it from outside. This property is called *immunity* and is specific to the phage type carried.

Another interesting feature of the λ system is that, in addition to negative regulation by the repressor, it involves positively acting regulatory elements. Although essentially all λ genes are blocked by immunity, most of them are only blocked indirectly. It is possible to switch on (or "transactivate") all the late-acting prophage genes without destroying immunity.⁶⁻⁸ The genes that can be transactivated are blocked by immunity because the repressor

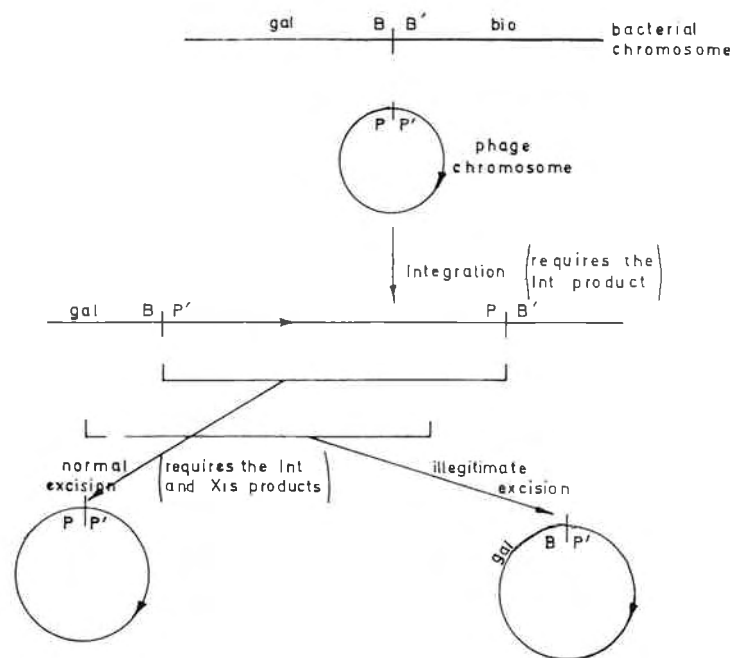


FIGURE 1. Integration and excision of λ . Illegitimate excision can produce a λ_{gal} particle carrying the bacterial *gal* gene. These particles have a BP' sequence instead of the PP' sequence found in normal phage. Only a short fragment of the bacterial chromosome is shown; in its entirety, it would be a circle some 100 times bigger than λ .

transactivation was rediscovered 20 years later in oncogenic viruses (and again baptized "transactivation.")

The λ -host system, simple in appearance, raises several interesting questions. What is the mechanism governing the decision between the lytic response and lysogenization? How can one reconcile the establishment of immunity, which switches off essentially all viral genes, and the integration of phage DNA, which requires the intervention of viral genes? And how is it that once immunity is established, gene *cI* continues to express itself, thus maintaining immunity? Sections II and III partly answer these questions.

II. INTEGRATION-EXCISION

A. NORMAL SITUATION

Integration of normal λ DNA into the genome of a nonlysogenic bacterium takes place, under the control of an "integrase" coded by gene *int* via site-specific recombination between a small region of the bacterial DNA, symbolized BB', and a region of the circularized phage DNA, symbolized PP' (Figure 1). As a result, the phage DNA, now called a prophage, is inserted between two hybrid sequences labeled BP' ("left") and PB' ("right").⁵

It was found that the reaction $BP' \times PB'$ (which leads to excision) requires the products of genes *int* and *xis*. Both genes can be expressed from a common promoter (P_L) under negative control of the repressor *cI* (in fact, a rather complex control, both direct and indirect). In addition, *int* can be expressed from a "private" promoter (pI), which requires the presence of the product of gene *cII*, itself under negative control of *cI* (again both direct and indirect).

We associate a logical variable r (for "repressor") with the product of gene cI , and variables c , i , and x with the products of genes cII , int and xis , respectively. The corresponding functions, as usual, are symbolized by capital letters (R , C , I , X). To describe the process of integration, we introduce a variable I that has the value 1 or 0 according to whether the phage DNA is integrated or not; the corresponding function L takes the value 1 if the phage DNA can be *expected* to be integrated in the immediate future, in other words, if the DNA is *not* integrated now, but conditions *permit* integration, or if it *is* integrated now and the conditions do *not* permit excision. In the present (normal) case, unintegrated phage DNA will become integrated if the product of gene int is present (\bar{i}), and integrated phage DNA will remain so if at least one of the products of genes int and xis is missing [$I(\bar{i} + \bar{x})$].

The simplified description given above can be formalized by the following logical relations:

$$R = 1$$

$$C = \bar{r}$$

$$I = c + \bar{r}$$

$$X = \bar{r}$$

$$L = \bar{i}i + I(\bar{i} + \bar{x})$$

In this description, the major simplification is that we consider the gene cI constitutive ($R = 1$). This simplification is acceptable if we provisionally consider only that fraction of the cells in which immunity will be established (the other cells will lyse). In these cells, we reason that the repressor will eventually be present (in fact, after a few minutes), and we can conveniently treat it as an input variable. In other words, we consider separately (in separate columns of the state table) the situation before ($r = 0$) and after ($r = 1$) the establishment of immunity; the 2^4 values of the variables c , i , x , and I are tabulated in lines, as usual.

For a normal (wild-type) phage infecting a nonlysogen, the situation is shown in Table 1. It can be seen in the compact state table that in the presence of immunity (right column), there are two stable states, $\textcircled{0000}$ and $\textcircled{0001}$, which correspond to unintegrated and integrated phage DNA, respectively. The other functions are off and their products are absent, as expected once immunity has been established for some time. In contrast, in the absence of immunity (left column), there are *no* stable states; instead, the system eventually reaches a two-state cycle, $1110 \rightleftharpoons 1111$. Since there is no immunity, the genes C , I , and X are on ($C = I = X = 1$) and their products have appeared ($c = i = x = 1$). When the phage DNA is unintegrated, it will tend to integrate because the int product is present, and when it is integrated, it will tend to be excised because the xis product is also present.

In practice, immunity can appear at any stage between the initial state $\bar{0}\bar{0}\bar{0}\bar{0}$ and the oscillating situation $111\bar{0} \rightleftharpoons 111\bar{1}$. According to the case, the system will end up in state $\textcircled{0001}$ or $\textcircled{0000}$. In the first situation, the phage DNA is integrated as a prophage, then immunity is established and we have a stable lysogen. In the second situation, the phage DNA is not integrated. Since immunity is established, the phage replication functions are repressed and the phage genome will be diluted out with bacterial growth. Such a cell will give rise to a population of nonlysogenic bacteria containing a single cell with an unintegrated phage DNA molecule. This cell will be immune, although it is not a stable lysogen. Since the

TABLE 1
Normal Integration

Complete state table			Compact state table	
	$r = 0$ (immunity absent)	$r = 1$ (immunity present)	$r = 0$ (immunity absent)	$r = 1$ (immunity present)
<i>cixl</i>	<i>CIXL</i>	<i>CIXL</i>		
0000	1110	0000	$\bar{0}\bar{0}\bar{0}\bar{0}$	(0000)
0001	1111	0001	$\bar{0}\bar{0}\bar{0}1$	(0001)
0011	1111	0001	$\bar{0}\bar{0}11$	00 $\bar{1}1$
0010	1110	0000	$\bar{0}\bar{0}10$	00 $\bar{1}0$
0110	1111	0001	$\bar{0}11\bar{0}$	0 $\bar{1}\bar{1}\bar{0}$
0111	1110	0000	$\bar{0}11\bar{1}$	0 $\bar{1}\bar{1}\bar{1}$
0101	1111	0001	$\bar{0}1\bar{0}1$	0 $\bar{1}01$
0100	1111	0001	$\bar{0}1\bar{0}\bar{0}$	0 $\bar{1}0\bar{0}$
1100	1111	0101	11 $\bar{0}\bar{0}$	$\bar{1}10\bar{0}$
1101	1111	0101	11 $\bar{0}1$	$\bar{1}101$
1111	1110	0100	111 $\bar{1}$	$\bar{1}\bar{1}\bar{1}\bar{1}$
1110	1111	0101	111 $\bar{0}$	$\bar{1}\bar{1}\bar{1}\bar{0}$
1010	1110	0100	1 $\bar{0}10$	$\bar{1}\bar{0}\bar{1}0$
1011	1111	0101	1 $\bar{0}11$	$\bar{1}\bar{0}\bar{1}1$
1001	1111	0101	1 $\bar{0}\bar{0}1$	$\bar{1}\bar{0}\bar{0}1$
1000	1110	0100	1 $\bar{0}\bar{0}\bar{0}$	$\bar{1}\bar{0}\bar{0}\bar{0}$

phage DNA is not integrated, it will not be replicated and is thus transmitted to only one of the daughter cells at division. The other daughter cell will still be immune for a time since the repressor is stable, but further growth will dilute the repressor below its threshold concentration, and immunity will ultimately be lost. This situation is called "abortive lysogeny".

It is interesting to note that the establishment of immunity is not necessarily accompanied by integration of the phage DNA into the bacterial chromosome. In fact, the λ circuitry is "wired" to avoid abortive lysogeny. The final result — integration or not — depends on the relative values of certain time delays. Without going into details, the general principle is that as long as both *int* and *xis* products are present, the process of integration-excision can operate in either direction; the disappearance of *int* would prevent both integration and excision, thus freezing the situation, whereas the disappearance of *xis* would block excision without preventing further integration. In actual fact, as pointed out by

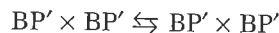
Weisberg and Gottesman,¹⁰ the half-life of the *xis* product is shorter than that of the *int* product. In addition, the establishment of immunity blocks gene *xis* (and *cII*) directly, whereas gene *int* is still expressed as long as there is *cII* product left. This explains the prevalence of integration over excision (and the avoidance of abortive lysogeny) in the case of normal λ infecting a nonlysogen.

There are, however, situations in which the conditions of integration and excision are different.

B. INTEGRATION OF λ_{gal} TO THE LEFT OF AN INTEGRATED PHAGE

Prophage excision is normally the reverse of integration: the left and right hybrid sites BP' and PB' flanking the prophage recombine to reconstitute a circular λ molecule with a normal PP' site (Figure 1). Rarely, however, excision takes place elsewhere. Such an event can generate a λ_{gal} phage, in which the bacterial *gal* genes, situated just to the left of the attachment site (and coding for enzymes involved in galactose utilization), are incorporated into the phage. The λ_{gal} phage has a left hybrid attachment site BP' instead of the normal phage site PP' (Figure 1). It was observed that BP' and the normal bacterial site BB' do not interact efficiently. However, BP' readily interacts with the hybrid sites BP' and PB', which flank a normal integrated prophage. In fact, the frequency of integration of a λ_{gal} phage in a nonlysogen (site BB') is low, and it is increased considerably by the presence of a normal "helper" phage. The resulting lysogens carry both phage, with the λ_{gal} inserted to the left of the normal λ (in the left hybrid site BP'). The λ_{gal} phage can also integrate efficiently in a lysogen, provided the prophage has a different immunity from λ (λ immunity would block *cII* and *int* expression from the infecting λ_{gal} phage). In this case, too, the λ_{gal} integrates in the left hybrid site BP'.

Examining the interaction between BP' of the λ_{gal} and the BP' at the left of an integrated phage, it can be seen that the reactions leading to integration or excision are



One would expect the enzymatic requirements of the two reactions to be the same. It is indeed observed that in this case excision, like integration, requires *int* but not *xis*. The logical relation for *L* thus becomes $L = \bar{l}i + l\bar{i}$ (or $L = l \oplus i$, where \oplus is the exclusive "or"). This system is simpler than the normal one since *xis* is not involved:

$$R = 1$$

$$C = \bar{r}$$

$$I = c + \bar{r}$$

$$L = \bar{l}i + l\bar{i}$$

Table 2 gives the complete and compact state tables and Figure 2 gives the various possible pathways the system can follow. As in the preceding case, after the establishment of immunity ($r = 1$) there are two stable states, $\textcircled{000}$ and $\textcircled{001}$ (with the phage DNA unintegrated and integrated, respectively) whereas in the absence of immunity ($r = 0$) the system evolves toward the two-state cycle $11\bar{0} \rightleftharpoons 11\bar{1}$. The difference from the normal case is that

TABLE 2
Integration of λ_{gal} to the Left of a Prophage

Complete state table			Compact state table		
<i>cil</i>	<i>r</i> = 0	<i>r</i> = 1	<i>r</i> = 0	<i>r</i> = 1	
	(immunity absent)	(immunity present)	(immunity absent)	(immunity present)	
	<i>CIL</i>	<i>CIL</i>			
000	110	000	$\bar{0}\bar{0}\bar{0}$	$\textcircled{000}$	
001	111	001	$\bar{0}\bar{0}1$	$\textcircled{001}$	
011	110	000	$\bar{0}1\bar{1}$	$0\bar{1}\bar{1}$	
010	111	001	$\bar{0}1\bar{0}$	$0\bar{1}\bar{0}$	
110	111	011	$1\bar{1}\bar{0}$	$\bar{1}1\bar{0}$	
111	110	010	$1\bar{1}\bar{1}$	$\bar{1}1\bar{1}$	
101	111	011	$1\bar{0}1$	$\bar{1}\bar{0}1$	
100	110	010	$1\bar{0}\bar{0}$	$\bar{1}\bar{0}\bar{0}$	

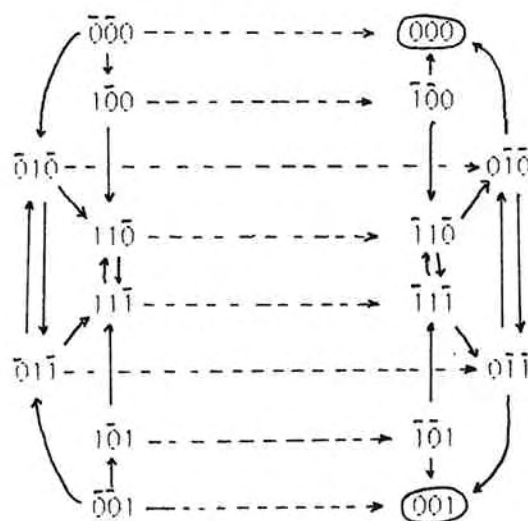
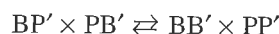
 $r = 0$ $r = 1$ 

FIGURE 2. Integration pathways of a λ_{gal} to the left of a prophage. The variables are listed in the order c, i, l , as in the text.

here there is not a race for the disappearance of the *int* and *xis* products. The probability of stable integration depends primarily on the relative rates of the integration and excision processes.

C. INTEGRATION OF λ_{gal} TO THE RIGHT OF AN INTEGRATED PHAGE

The right hybrid site of an integrated λ phage, PB' , can also interact with the λ_{gal} BP' site. In this case, the interactions are



This situation is exactly the reverse of the normal one: integration involves the interaction $BP' \times PB'$ whereas excision requires the interaction $BB' \times PP'$. One can thus surmise that integration of the λ_{gal} in the right hybrid site will require both *int* and *xis* products, whereas excision will require only *int*. This is indeed the case. The relation is

$$L = \bar{l}ix + \bar{l}\bar{i}$$

In the state table (not shown), one finds the same two stable states in the presence of immunity and the two-state cycle in its absence. Here, however, the short life span of the *xis* product acts in favor of excision, which explains why one does not find λ_{gal} integrated to the right of a prophage.

D. TO INTEGRATE OR NOT TO INTEGRATE?

The above formalizations are satisfying in the sense that, for appropriate values of the time delays, they predict the behavior that is actually found, for each situation, in the majority of cells. One may ask, however, why there are always some cells that behave differently. After all, if the delays are fixed, each infection should lead to the same result. The most likely answer, as discussed in Chapter 4, is that, in fact, the delays are not rigidly constant, but vary somewhat from cell to cell. When a distribution of the values of the time delays is introduced in a computer simulation of λ integration, it is easy to find parameter values yielding the percentage of integration actually found in the various cases.¹¹

III. THE CONTROL OF IMMUNITY

As shown by Jacob and Wollman¹² and Bertani,¹³ immunity in lysogens is ensured by a negative regulator, the repressor, encoded by a prophage gene, called *cI* in phage λ . The repressor recognizes specific DNA sequences (operators) located on either side of the *cI* gene and interacts with them to block — directly or indirectly — the expression of essentially all other viral genes. It was subsequently discovered that *cI*, which controls all other phage genes, is itself regulated. It can be expressed from two distinct promoters: p_{rm} (promoter for repressor maintenance), used for low, permanent synthesis, and p_{re} (promoter for repressor establishment), used for strong transient synthesis. The p_{rm} promoter is under positive control by *cI*. The p_{re} promoter is only functional in the presence of the positive regulator *cII*, which is itself under negative control by both *cI* and *cro*. It is also under positive control by the N protein, which is negatively regulated by *cI* and *cro* (see Ptashne³). The situation is clearly complex, involving a number of interacting feedback loops. Basically, however, *cII* acts as a starter, and the *cI* product, once present, continues to catalyze its own synthesis.

Despite the complexity, the essential features of the establishment of immunity can be

satisfactorily described as follows. Once gene *cII* is expressed, *cI* repressor is massively synthesized via p_{re} . As soon as enough *cI* product is present, it switches off *cII* expression and catalyzes its own continued synthesis via p_{rm} . However, immunity is not established in all cells (which is one of the features that make temperate phage interesting!). In a significant fraction of them, the process is prevented by the *cro* protein, which exerts a negative regulation on *cII* (and on other genes, including *cro* itself). This simplified scheme of the decision to establish immunity or not is used several times as an example in this book (Chapters 4, 14, and 23).

The Jacob group carried out a penetrating analysis of the control of immunity,¹⁴ using a highly simplified system. Their starting material was a λ mutant whose repressor is thermosensitive. When lysogens for this λcI_{ts} mutant are exposed to high temperature, the repressor is reversibly inactivated, all viral functions are derepressed, and the lytic cycle is triggered, resulting in phage multiplication and cell lysis. However, if appropriate viral genes are mutationally inactivated, the lysogens can tolerate the loss of immunity and continue growing at high temperature. It was this type of strain that was used to study the control of immunity.

If life were simple, one might expect such strains to be immune at low temperature and nonimmune at high temperature, *voilà tout*. But things are not so simple! If these strains are exposed *briefly* to high temperature and then returned to low temperature, they do indeed lose immunity, then recover it immediately. If however, the exposure to high temperature is *long* (several hours), the recovery of immunity on return to low temperature is no longer immediate, and, in fact, for some of the mutants studied, immunity was not recovered at all under these conditions.

The fact that immunity was not recovered immediately indicated that after a long exposure to high temperature, the cells were no longer synthesizing repressor (remember that the thermal inactivation of this mutant repressor is reversible). Thus, exposure of these lysogens to high temperature resulted not only in the inactivation of the repressor already present (and its ultimate dilution through growth), but also in a block of further repressor synthesis. The simplest hypothesis, proposed by Eisen et al., is that in this system the presence of active repressor is necessary for its own synthesis, i.e., repressor synthesis is autocatalytic. This positive loop was later demonstrated experimentally by Spiegelman et al.¹⁵

Let us first focus on the strains that fail to recover immunity, even a long time after the return to low temperature. The interest of this system is obvious: genetically identical bacteria growing in identical conditions (at low temperature) can persist in either of two stable states, nonimmune or immune, according to their past history (exposed or not to high temperature). Formally, these strains can be accounted for simply in terms of the autocatalytic character of *cI* synthesis. Letting *r* represent *cI* concentration and *t* the temperature, the logical relation is

$$R = r\bar{t}$$

which means that the *cI* gene is expressed iff there is active repressor present, i.e., iff there is repressor *and* the temperature is low. This gives the following state table:

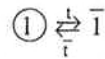
Complete state table

<i>r</i>	<i>t</i>	
	<i>t</i> = 0	<i>t</i> = 1
<i>R</i>	<i>R</i>	<i>R</i>
0	0	0
1	1	0

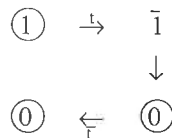
Compact state table

<i>r</i>	
<i>t</i> = 0	<i>t</i> = 1
⓪	⓪
①	1̄

Starting with a culture in the immune state ① at low temperature and shifting it to high temperature, the repressor is inactivated and its synthesis therefore stops ($R = 0$). If the culture is returned to low temperature before the preexisting repressor disappears, it will renature and again catalyze repressor synthesis; in other words, a *brief* stay at high temperature means "shorter than t_r ". The pathway is

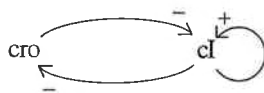


If, however, the stay at high temperatures is *long*, i.e., there will no longer be any repressor left to renature on return to low temperature, and synthesis will not be able to resume; the system is trapped in the state 0. The pathway is



Let us now examine the situation in those mutants that do recover immunity, albeit slowly, if the time at high temperature was long. In these strains (unlike the previous ones), either repressor can be synthesized in the total absence of preexisting repressor or some residual repressor synthesis takes place at high temperature. In the latter case, we should further postulate that the residual repressor present at high temperature is not enough to restore immunity on return to low temperature, but merely to reinitiate repressor synthesis. These considerations let Eisen et al. to postulate (and later to establish genetically) the existence of a gene, *cro*, whose product exerts a negative control on the synthesis of *cI* and that is itself under negative control by *cI*, like the other λ genes. The strains that fail to recover immunity are *cro*⁺, whereas those that do recover immunity are *cro*⁻.

The positive loop between *cI* and *cro*, which provides *indirect* autocatalysis of *cI*, was discussed in Chapter 17. Knowing that it exists, we must now go back one step and ask whether it is still logically necessary to postulate a *direct* positive control of *cI* on itself. The fact that this loop has been demonstrated experimentally does not imply that it is necessary to account for the observed behavior: it could be a case of "belt and suspenders". However, it is, in fact, essential. In the absence of the *cI*⁺ loop, we cannot account for the block of *cI* expression at high temperature in the *cro*⁻ strains (in the *cro*⁺ strains, of course, the *cro* product is sufficient to block *cI*). For this reason, we assume *both* positive loops have to be taken into consideration:



We must now make a more precise analysis of the interactions among the elements of our system. Let us assume that no repressor whatsoever is synthesized at high temperature, but that repressor *can* be formed in the absence of preexisting repressor, provided no *cro* product is present. This can be described by the relation:

$$R = (r + \bar{d})\bar{t}$$

where d represents the concentration of *cro* product, r that of repressor, and t represents the temperature. Letting g_D indicate the state of the *cro* gene (normal or inactive), the relation for *cro* expression is

$$D = g_d(\bar{r} + t)$$

which simply says that the *cro* gene is under negative control of the thermosensitive repressor *cI*. The state table is

Complete table					Compact table			
rd	cro^+		cro^-		rd	cro^+		cro^-
	$t=0$	$t=1$	$t=0$	$t=1$		$t=0$	$t=1$	
00	11	01	10	00	00	00	00	00
01	01	01	—	—	01	01	01	01
11	10	01	—	—	11	11	11	11
10	10	01	10	00	10	10	10	10

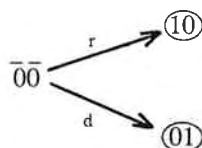
It accounts for the results of Eisen et al.¹⁴ and, in particular, for the nonrecovery of immunity in *cro*⁺ strains and its recovery in *cro*⁻ strains.

IV. FEEDBACK BETWEEN MODELS AND EXPERIMENTS

The above results were deduced from studies of defective lysogens. In addition to a thermosensitive mutation in the *cI* gene, the prophages carried mutations inactivating the essential positive control gene *N* and the replication functions. From the state table, can we predict anything about the behavior of λN^-cro^+ and λN^-cro^- phage after infection of nonlysogens? Indeed we can, simply by taking 00 as initial state (*cI* and *cro* both absent).

In fact, the λN^-cro^+ phage had already been studied before the discovery of *cro*. Gene *N* codes for a positive regulator involved directly or indirectly in the expression of all λ genes⁶ except *cro* and *N* itself. Thus a λN^- mutant cannot carry out a lytic cycle, integrate, or establish immunity. However, it can replicate slowly and maintain itself as an autonomous plasmid.¹⁶ An easy way to detect this plasmid is to use a λN^-gal variant in a *gal*⁻ strain, where its presence confers a *Gal*⁺ phenotype.

Starting from 00, a λN^-cro^+ phage can in principle go to either 10 or 01:



However, since it takes only a few minutes before the effect of gene *cro* is detected, whereas it takes over 30 min before a λN^-cro^- lysogen recovers immunity, we conclude that the delay t_d is much shorter than the delay t_r . This accounts for the fact that indeed very few bacteria become immune following infection with λN^-cro^+ ; in almost all of the cells, λN^-cro^+ is established as a plasmid.

If we look at the situation following infection with λN^-cro^- , we see the only predicted pathway is

$$\bar{0}0 \rightarrow \textcircled{10}$$

In the preceding case, this pathway was not normally followed because there was an alternative, faster pathway. If the model is correct, we expect efficient establishment of immunity after infection with λN^-cro^- . In the absence of phage integration, of course, this would result in loss of the phage by dilution. Experimentally, it is indeed found that λN^-cro^- never establishes itself as a plasmid. Instead, the infected cells become stable lysogens, which implies systematic establishment of immunity, as expected, and efficient integration as well.

It is known that gene *cII* is poorly expressed in λN^- mutants. In fact, the simple model presented here does not take gene *cII* into account for this reason. We had to check whether this assumption is justified or whether *cII*, although only slightly expressed, plays a role in the establishment of immunity by λN^-cro^- . A simple test consisted in comparing the behavior of a $\lambda N^-cro^-cII^-$ phage with its *cII*⁺ homologue. It was found that in spite of its *N*⁻ and *cII*⁻ character, $\lambda N^-cro^-cII^-$ does lysogenize at a significant rate, although several times less efficiently than its *cII*⁺ counterpart. Direct measurements of the establishment of immunity, as well as the almost complete absence of the establishment of the phage DNA as a plasmid, indicate that, in fact, immunity is established in most cells and that the difference between the *cII*⁺ and *cII*⁻ phage is at the level of integration. Moreover, a lysogen carrying a λN^-cro^- prophage deleted for gene *cII* does recover immunity, again showing that *cII* is not involved.

These results are in essential agreement with the model as regards the establishment of immunity. If we also wish to account for the frequency of integration, gene *int* must be included in the model. In fact, this aspect was studied separately from immunity in Section 2, where we saw that gene *cII* indeed plays a major role in the expression of the *int* gene. Remember, too, that λgal requires a resident prophage to integrate. For this reason, we used strains that were lysogenic for a closely related heteroimmune phage. Integration to the left of this prophage requires only the *int* product.

The above results are described in more detail in Thomas et al.¹⁷ In this work, there was constant feedback between the models and the experimentation.



For the record, it may be interesting to mention that several people congratulated us for the remarkable fit between the predictions of the model and the experimental results. This was taken as proof of the excellence of the method. We feel that the method would have been just as good and useful if it had shown us that the model was wrong.

V. GENE DOSAGE, NEGATIVE LOOPS, AND *cro*

The steady concentration of the product of an unregulated gene is the ratio of its synthesis and decay rate constants, k/k_- , and if a cell possesses m copies of the gene, the steady concentration of the product will be mk/k_- . This proportionality is called *gene dosage*. The concentration of such products would be expected to increase suddenly when the gene is replicated. For certain gene products, this sort of fluctuation is undesirable. In particular, for viruses, the replication involves not a simple doubling in gene copy number, but a 100-fold

or more increase, often within less than 30 min. In these cases, **gene dosage can be abolished by a negative loop on the gene.** Although it is clear that any negative regulation can reduce the expression of each copy of the gene, it is perhaps less obvious that the *proportionality* can be eliminated by negative autoregulation. The reason, of course, is that any increase in the concentration of the product causes a reduction in its rate of synthesis via the negative feedback loop.

Let us analyze this quantitatively for a gene \underline{X} present at m copies per cell. We will compare the effect of (1) a repressor \underline{Z} , which reduces its rate of expression, with that of (2) a negative feedback loop on the gene, whereby \underline{x} reduces its own expression. In the first case, the differential equation for \underline{x} expression is

$$dx/dt = mk(\vartheta^n)/(\vartheta^n + z^n) - k_-x \quad (i)$$

At steady state, we have the relation:

$$x = m(k/k_-)(\vartheta^n)/(\vartheta^n + z^n)$$

Letting x_1 represent the steady-state concentration of \underline{x} for 1 copy of the gene, we have:

$$x_m/x_1 = m$$

In other words, although the rate of expression of \underline{x} is reduced by a factor $(\vartheta^n)/(\vartheta^n + z^n)$, which depends on the concentration of \underline{z} , gene dosage persists.

In the second case, we have

$$dx/dt = mk(\vartheta_x^n)/(\vartheta_x^n + x^n) - k_-x \quad (ii)$$

and at steady state, we have the equation:

$$x = m(k/k_-)(\vartheta_x^n)/(\vartheta_x^n + x^n)$$

in which x has a well-defined value. Assuming homeostasis is effective — i.e., that k/k_- is sufficiently greater than ϑ_x — the steady state value of x will be arbitrarily close to ϑ_x for sufficiently high n . Thus, for high values of n , we have

$$x_m/x_1 \approx 1$$

and gene dosage is abolished.

These considerations are probably relevant for the negative control exerted by the λ *cro* protein on its own operon. In this operon are found the *cII* regulatory gene and the *O* and *P* genes, whose products are required for λ DNA replication. It must be assumed that the level of expression of *O* and *P* from a single λ DNA molecule is sufficient since, otherwise, infection by a single phage particle could not lead to replication and lytic growth, contrary to observation. If there were no autoregulation of the operon, these genes would be expressed at a 100-fold higher rate when the cell contains 100 copies of the phage genome. This would probably be useless for the *O* and *P* proteins and might well be lethal in the case of the *cII* protein. Thus, the negative control exerted by *cro* on its own operon has the effect of providing an essentially constant rate of expression of genes *cII*, *O*, and *P* (and *cro* itself), irrespective of the number of copies of λ DNA present.

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Chapter 21

NEURONAL NETWORKS**TABLE OF CONTENTS**

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I. INTRODUCTION

Neurobiology is *par excellence* the domain of complex networks. The nervous system collects data from outside via the sense organs (*inputs* of the system) and, in response, sends orders to muscles, glands, etc. (*outputs* of the system). Much of the system is an exceedingly complex network involving refined feedback interactions that can be treated in terms of *internal variables and functions*.

As is well known, the elementary unit in the nervous system is a cell (the neuron), which, in addition to many receptor extensions (the dendrites), has an emitter extension (the axon), a fiber that may be 1 m long or more and whose distal end is branched. Neurons generate waves of impulses that propagate along the axon and are transmitted from a ramification of the axon to a dendrite of another neuron (or to a muscle, etc.) via a *synaptic junction*. Whereas the transmission along the axon is electrical (a wave of membrane depolarization), the transmission at a synaptic junction is chemical, via a neurotransmitter.

We know there are some genes that function unless a repressor prevents them from doing so (negative control), and others that function only in the presence of an activator (positive control). Similarly, there are neurons "provided with tonic excitation"⁶ that emit impulses unless they are prevented from doing so by the action of one or more presynaptic inhibitory neurons, whereas other neurons are active only if they have received an order from an activating presynaptic neuron. In the first case, the neuron will not emit impulses as long as the presynaptic inhibitory neuron is active: when the inhibitory neuron becomes silent, the neuron in question starts emitting impulses after a lag, necessary for the "recovery from inhibition".

In what follows, we will try to avoid technical terms as much as possible and, instead, speak in terms of logical networks, as in the other chapters.

It must be remembered that the nervous system of higher organisms is a huge network that may comprise more than 10^{10} elements. With this in mind, it may seem futile to study the behavior of networks comprising only a small number of neurons. However, there are enormous sets of neurons that behave more or less collectively and can at least provisionally be treated as a unit. In addition, as long as we do not understand the behavior of small neuron networks, there is not the slightest hope of understanding the operation of more complex circuits.

Logical modelization of neuron networks was proposed and already used long ago by Rashevsky,¹ McCulloch and Pitts,² Adam,³ and others. Rashevsky relates the situation of a neuron at time t to that of antecedent neurons at time $t - \Delta t$, themselves related to the situation of their own antecedents at time $t - 2\Delta t$, etc. Adam, in association with Kling and Székely,⁴ uses the terminology of graph theory: "we use the terminology 'graph', 'vertex' and 'edge' for 'net', 'neuron' and 'axon', respectively". In their logical description, inhibition acts instantaneously (i.e., the "off" delays are nil) and recovery from inhibition has the same delay τ for each neuron.

We are not neurobiologists, and this chapter should not be considered more than "an exercise with neurons" (cf. Thomas⁵). The exercise will be centered around a remarkable piece of work by Friesen and Stent⁶ on the locomotion of the leech. With fascinating feedback between cytological observation, experimentation, and theory, they reached the conclusion that much of the periodic locomotion of this worm depends on a small number of neurons, four in each ganglion, of which there are two in each segment. The theoretical aspects are influenced by earlier work by Kling and Székely, who put special emphasis on the idea that periodic activity of neurons does not necessarily require that certain neurons have *intrinsic* periodic activity, but, rather, that it can be accounted for by *recurrent cyclic inhibition*

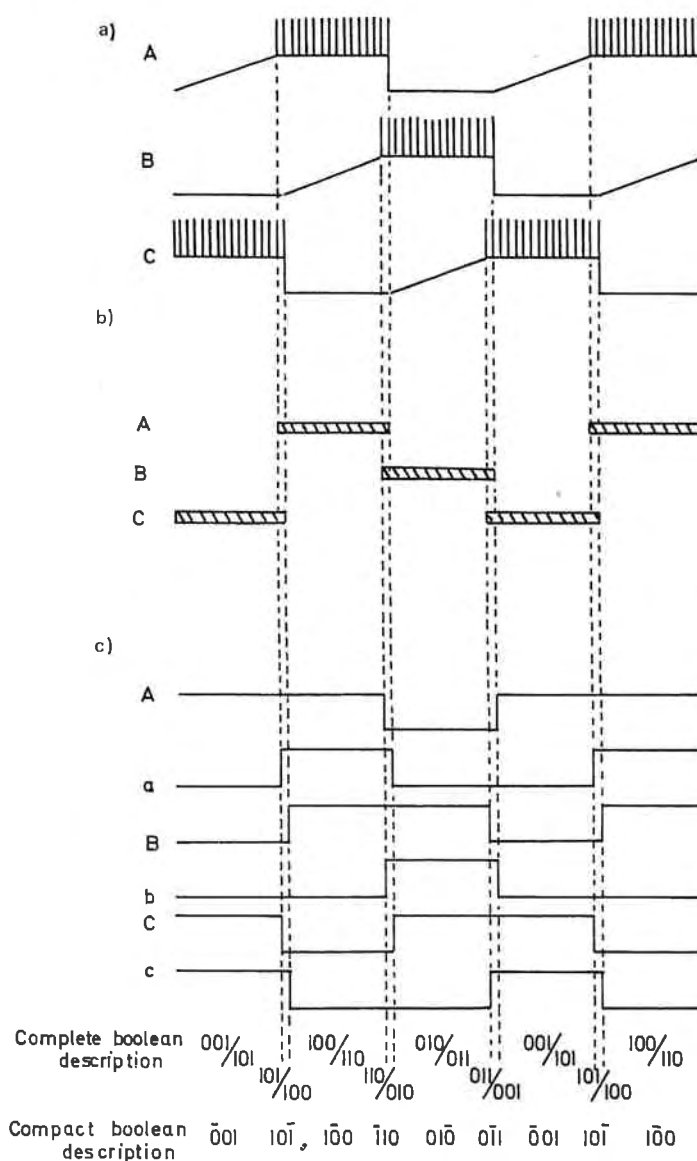


FIGURE 1. (a) Traces representing the membrane potential and impulse activity of individual neurons; (b) phase diagram; (c) representation of the values of the boolean function and variable associated with each neuron.



involving an odd number of elements (see Section II). Note that this structure is a typical negative loop.

II. A THREE-NEURON OSCILLATING NETWORK

Let us first quote Friesen and Stent's description⁶ of the three-element loop:

This network consists of an inhibitory ring formed by three tonically excited neurons (A–C), each of which makes inhibitory synaptic contact with and receives inhibitory synaptic input from one other cell. If, as indicated in Figure 1a, Cell C happens to be in a depolarized, impulse-generating state, its postsynaptic cell, B, must be in a hyperpolarized, inactive state, while its presynaptic cell, A, is recovering from past inhibition. As soon as Cell A

has recovered from inhibition and reached its impulse generation threshold, Cell C becomes inhibited, thus disinhibiting Cell B and allowing the latter to enter its recovery phase. Once Cell B has recovered, it inhibits Cell A, thus allowing Cell C to begin recovery; and once Cell C has recovered, so that Cell B enters its inactive phase and Cell A its recovery phase, one cycle of the oscillation has been completed.

Situations can be described by *phase* diagrams (Figure 1b) in which rectangles indicate at each time which neurons are "on" (i.e., emitting impulses). A more sophisticated description ("trace") commonly used (Figure 1a) indicates whether a neuron is emitting impulses (symbol ) and, if not, whether it is inactive or "recovering from inhibition" (symbol ) and about to start emitting impulses.

Quite naturally, we associate with each neuron a logical variable (a, b, c, \dots) whose value is 1 if the neuron is emitting impulses, 0 if not, and a logical function (A, B, C, \dots) that takes the value 1 if there is an order to emit impulses, 0 if there is no such order (Figure 1c and d). Thus, the successive logical states of a neuron are

1. 0/0: no impulses, no order to emit them
2. 0/1: no impulses, but an order to emit them
3. 1/1: order executed (impulses being emitted) and still valid
4. 1/0: impulses still being emitted, but there is an order to stop
5. 0/0: again, no impulses (order to stop emitting has been executed)

Note that Friesen and Stent, as well as Kling and Székely, assume that the order to inhibit the emission of impulses is executed immediately, unless it comes from a neuron located in a different ganglion. Thus, the "off" delays are nil and, in practice, the system proceeds directly from 1/1 to 0/0 without delay.

If we write the (naïve) logical relation corresponding to this loop:

$$A = \bar{b}$$

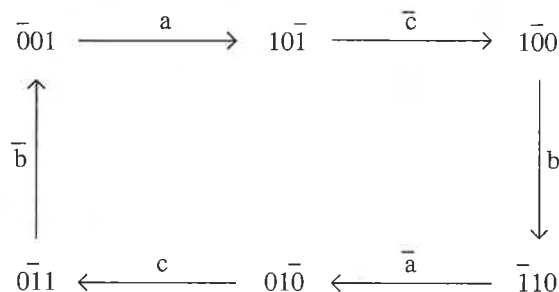
$$B = \bar{c}$$

$$C = \bar{a}$$

we immediately obtain the state table:

a	b	c	A	B	C
$\bar{0}$	$\bar{0}$	$\bar{0}$	1	1	1
$\bar{0}$	0	1	1	0	1
0	$\bar{1}$	1	0	0	1
0	1	$\bar{0}$	0	1	1
$\bar{1}$	1	0	0	1	0
$\bar{1}$	$\bar{1}$	$\bar{1}$	0	0	0
1	0	$\bar{1}$	1	0	0
1	$\bar{0}$	0	1	1	0

from which we derive the cyclic behavior:



Another example is treated inductively (from behavior to logical structure) in Chapter 5, Section III.

III. LEECH LOCOMOTION

Friesen and Stent were able to derive models involving up to four ganglia, each with four crucial neurons. The dynamics of these models was checked by the authors using "neuromimes", which are sophisticated analogic devices. In view of the relative complexity of these devices, the number of neuromimes available was limited (eight) and when simulating systems involving more than two ganglia, the authors had to replace some indirect connections by direct ones (in order to lower the number of neurons considered).

As our logical analysis is not subject to this type of limitation, it was tempting to apply it to these models. As a first step, we formalized three variants of the Friesen-Stent model involving eight neurons from two ganglia and checked whether our simulations fit those of Friesen and Stent with their neuromimes. Next, we turned to a more complex system comprising 12 neurons belonging to three ganglia. For this system, we had the experimental phase diagram, kindly sent by Friesen and Stent, and their model given in Reference 6, Figure 10. The simulation with neuromimes (using a reduced model with eight neurons) shows that the model meets most, but not all, features of the experimental phase diagram. We can now ask: (1) What are the simplest connections among the 12 neurons that would exactly reproduce the phase diagram?, (2) What constraints must be fulfilled to permit this exact sequence?, and (3) Are any of these constraints contradictory with the model proposed by Friesen and Stent? If so, it would indicate where the model should be modified.

We thus first formalized the three variants of a model provided by Friesen and Stent, involving eight neurons from two ganglia. The diagram (Figure 2) is, in fact, a graph of interactions in which certain details remain ambiguous. The "inhibitory connection" (symbol $\text{---}\bullet\text{---}$) corresponds to our negative interaction ($\text{---}\neg\text{---}$). The principal ambiguity is the precise interpretation of the "rectifying electrical junction" (symbol $\text{---}\blacktriangleright\text{---}$). The three variants reflect different interpretations of this connection. These variants, discussed in Friesen and Stent's paper⁶ (p. 36), differ according to the "major" or "minor" character of certain inhibitory connections, i.e., according to whether one inhibitor connection suffices to impose its effect or whether two or more presynaptic cells must cooperate. The three variants

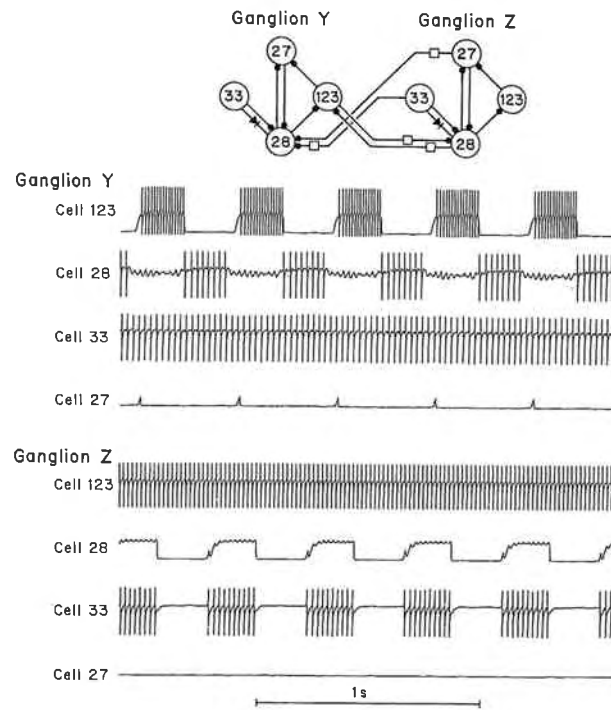


FIGURE 2. Oscillations of the full interneuronal network in two interconnected ganglia, Y and Z, of which Z is the rearmost of the chain. Impulse bursts were generated by eight neuromimes connected according to the circuit shown in the insert. The intraganglionic inhibitory connection from Cell 33 to Cell 28 is major in ganglion Z and minor in ganglion Y. All other connections are major. The rectifying electrical junction between Cells 33 and 28 is adjusted to be of such a strength that all major inhibitory inputs to Cell 28, except that provided by Cell 33 itself and its homologs, polarize Cell 33 beyond the impulse threshold potential. $H = 80$ ms. The free-running impulse frequency is about 80 Hz. (From Friesen, W. O. and Stent, G. S., Fig. 9 in *Biol. Cybern.*, 28, 27, 1977. With permission.)

can be formalized:

- | | | | | | |
|-----|--------------------------------------|-----|---|-----|---|
| (1) | $A = \bar{b}\bar{b}'$ | (2) | $A = \bar{b}\bar{b}'$ | (3) | $A = \bar{b}\bar{b}'$ |
| | $B = \bar{c}\bar{c}'\bar{d}\bar{d}'$ | | $B = \bar{c}\bar{c}'(\bar{d} + \bar{d}')$ | | $B = \bar{c}\bar{c}'(\bar{d} + \bar{d}')$ |
| | $D = \bar{c}\bar{c}'\bar{d}'$ | | $D = \bar{c}\bar{c}'$ | | $D = \bar{c}\bar{c}'$ |
| | $C = \bar{a}\bar{b}$ | | $C = \bar{a}\bar{b}$ | | $C = \bar{a}\bar{b}$ |
| | $A' = \bar{b}'$ | | $A' = \bar{b}'$ | | $A' = \bar{b}'$ |
| | $B' = \bar{a}\bar{c}'\bar{d}'$ | | $B' = \bar{a}\bar{c}'$ | | $B' = \bar{a}\bar{c}'\bar{d}'$ |
| | $D' = \bar{a}\bar{c}'$ | | $D' = \bar{a}\bar{c}'$ | | $D' = \bar{a}\bar{c}'$ |
| | $C' = \bar{a}'\bar{b}'$ | | $C' = \bar{a}'\bar{b}'$ | | $C' = \bar{a}'\bar{b}'$ |

in which a , b , d , and c symbolize neurons 123, 28, 33, and 27, respectively; a and a' refer to homologous neurons in two different leech segments. Submodel 3 is the variant used by Friesen and Stent in their Figure 9. Friesen wrote: "I have carefully checked your logical statements and found that they describe precisely (and concisely) the neuronal interconnec-

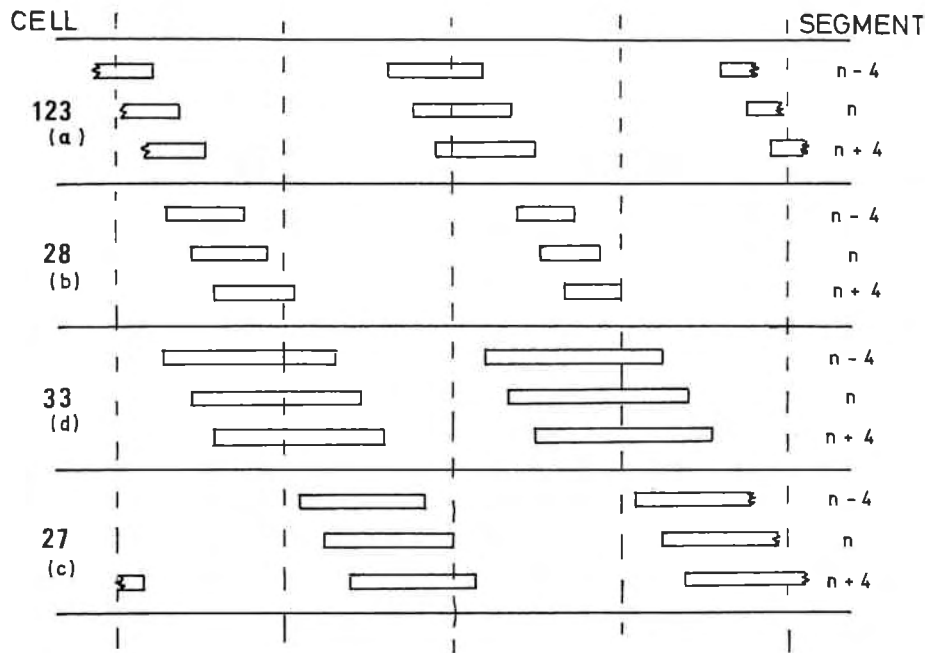


FIGURE 3. Experimental phase diagram (provided by W. O. Friesen) showing, as a function of time, the activities of 12 neurons from three ganglia.

tions illustrated in Figure 9 of our 1977 Biological Cybernetics paper." Moreover, we checked that, with the time delays (including the intersegmental impulse conduction delays), simulations on the logical machine "Delphin"⁷ or with computer programs⁸ reproduce the essential features of the neuromime simulation.

More specifically, submodel 1 stabilizes at the stable state 10101000, in which neurons *a*, *d*, and *a'* are on and the others off. Submodel 2 stabilizes at the stable state 00010110, in which neurons *c*, *b'*, and *d'* are on and the others off. Submodel 3 leads to a situation in which neurons *d* and *a'* are on; *c*, *b'*, and *c'* are off; and the others oscillate (as in Figure 2). In all three cases, our simulations agree with those using neuromimes.

We wanted to know what the behavior of these models would be if, instead of imposing fixed values to the time delays, we gave each delay an average value and a distribution. The results were extremely interesting and can be briefly described as follows.

1. For each submodel, the most frequent pathway was that already found with the time delays proposed by Friesen and Stent.
2. Whereas in the simulation with fixed delays, submodels 1 and 2 fail to oscillate and the additional modification leading to submodel 3 is required to obtain oscillations, with the distributed delay values, even submodels 1 and 2 give oscillations. Furthermore, the oscillation found most frequently in submodel 2 is that of Figure 2, originally given by submodel 3.

In Figure 3, we find an experimental phase diagram, kindly provided by Friesen. In Figure 4, there is a model including 12 neurons from three ganglia. For lack of enough neuromimes, the authors have considerably simplified the circuit for their simulations (although "without loss of verisimilitude"). The results of the simulations (Figure 4) partly fit the

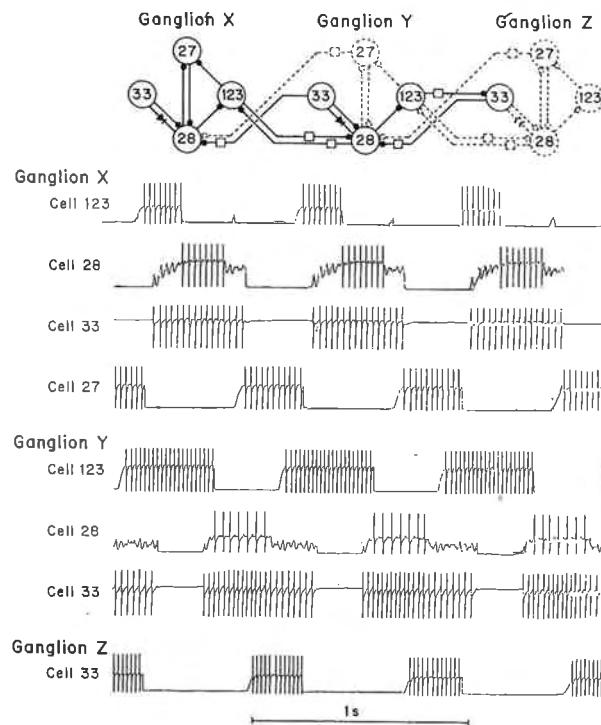


FIGURE 4. Oscillation of the full interneuronal network in three ganglia. X, Y, and Z impulse bursts were generated by eight neuromimes connected according to the circuit shown in the insert. Neuromimes representing the nonoscillatory Cells 28, 123, and 27 of the rearmost ganglion Y have been omitted from the circuit since they make no major contribution to the rhythm generation. The indirect major connection of Cell 123 of ganglion Y to Cell 33 of ganglion Z via Cell 28 has been replaced without loss of verisimilitude by a direct, major inhibitory link. $H = 80$ ms. Free-running impulse frequency about 80 Hz. (From Friesen, W. O. and Stent, G. S., Fig. 10 in *Biol. Cybern.*, 28, 27, 1977. With permission.)

experimental phase diagram in the sense that all neurons oscillate. However, the order of the commutations is not entirely correct.

Taking into account Friesen and Stent's remarks about the "major" or "minor" character of connections, and the properties of "rectifying electrical junctions" (Reference 6), a precise graph of interactions can be derived from the scheme of Figure 4, and the full model can be described by the logical relations:

$$\begin{array}{lll}
 A = \bar{b}\bar{b}' & A' = \bar{b}'\bar{b}'' & A'' = \bar{b}'' \\
 B = \bar{c}\bar{c}'(\bar{d} + \bar{d}') & B' = \bar{a}\bar{c}'\bar{c}''(\bar{d}' + \bar{d}'') & B'' = \bar{a}'\bar{c}''\bar{d}'' \\
 D = \bar{c}\bar{c}' & D' = \bar{a}\bar{c}'\bar{c}'' & D'' = \bar{a}'\bar{c}'' \\
 C = \bar{a}\bar{b} & C' = \bar{a}'\bar{b}' & C'' = \bar{a}''\bar{b}''
 \end{array}$$

in which a , b , d , and c symbolize neurons 123, 28, 33, and 27, respectively.

Using the time delays proposed by Friesen and Stent (including intersegmental impulse conduction delays), simulations on the logical machine "Delphin"⁷ or with computer programs,⁸ as expected, give results essentially identical to those obtained in Figure 4 with neuromimes.

As already mentioned, this resembles the experimental phase diagram, but does not fit perfectly. One can, in fact, derive simple connections that permit or impose a sequence exactly coincident with the experimental phase diagram. However, this represents a choice among many possible solutions that may be very different from the real one.

It is probably more interesting to identify those constraints that must be satisfied to permit the experimentally observed sequence and check whether any features of the model violate these constraints. One way (simplified from the methods described in Chapter 5) consists in comparing the states of the system just before a variable (say, a), is switched on and just before it is switched off. At least one of the differences is required in order to have $A = 1$ in the first case and $A = 0$ in the second case. For example, the states immediately preceding the lighting and extinction of variable a are, respectively:

	a	a'	a''	b	b'	b''	d	d'	d''	c	c'	c''
State just before variable a is switched on	0	0	0	0	0	0	0	0	1	1	1	1
State just before variable a is switched off	1	1	1	0	0	0	0	0	0	0	0	0

We conclude that one or more of the orders \bar{a} , \bar{a}' , \bar{a}'' , d'' , c , c' , and c'' must appear in the expression of function A . This analysis has been done for each of the 12 functions of the system, starting from the experimental phase diagram. When we compare these requirements with the logical description of the author's model, we find that there is no problem for 9 of the 12 functions. However, for three functions (A , A' , A''), none of the required connections are present in the model. We infer that the discordances between the predictions of the model and the phase diagram could be avoided by changing the model at the level of these functions. More specifically, a comparison of the constraints on functions A , A' , and A'' indicates that a link, $c \rightarrow +a$, in each ganglion would permit the proper sequence.

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Chapter 22

A MODEL ANALYSIS OF THE IMMUNE RESPONSE**M. Kaufman, R. D'Ari, and R. Thomas****TABLE OF CONTENTS**

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I. INTRODUCTION

This chapter summarizes work published by Kaufman, Urbain, and Thomas,¹ Kaufman and Thomas,² and Kaufman.³ In short, it gives an interpretation of immune memory and paralysis in terms of transitions among multiple steady states. There is not room here for a course on immunology. This introduction simply tries to make our approach understandable to nonbiologists.

The immune system of vertebrates responds to the introduction of foreign macromolecules (notably, proteins and polysaccharides), called *antigens* in this respect, by the production of specific proteins called *antibodies* or immunoglobulins. Antibodies combine with the antigen against which they have been raised, neutralizing it, and thus contribute significantly to the defense of the organism.

The immune system is one of our principal defenses against invading microbes. Even in cases in which the response was too slow to prevent the disease, it helps combat it. Furthermore, after the first encountering of an antigen, the system becomes endowed with memory. This is the well-known phenomenon of "immunity" to diseases one has already had; it explains why a given individual seldom catches measles, mumps, etc. a second time.

The immune response has been widely exploited by the medical profession. Vaccination is basically a way of tricking the immune system to produce antibodies against a specific microbe *before* the microbe infects the individual. The principle is to inject an antigen, the vaccine, which is so similar to the microbe that some of the antivaccine antibodies will crossreact with the microbe.

The immune response is also used in disease detection. It is easy to test whether an individual is producing antibodies of a certain specificity. If not, one can assume he has never been exposed to that particular antigen (generally a microbe). A positive response means he has been exposed, although, in view of the self-propagating nature of antibody production, it does not tell us whether the antigen (microbe) is still present. This is the basis of standard tests for detecting such diseases as syphilis and AIDS.

A. ANTIBODY DIVERSITY

One astonishing aspect of the immune response is the fact that we (vertebrates) are able to produce specific antibodies against practically any antigen, even exotic ones that have surely never been encountered before by our ancestors. For example, as pointed out by Urbain,⁴ we could very well produce antibodies specifically directed against crocodile tear lysozyme. Moreover, a given antigen can be recognized by different specific antibodies. This is due to the complex structure of most antigen molecules; each specific antibody recognizes only a small portion of the antigen. Antigenic specificity can be achieved in a number of different ways. Without going into detail, let us mention that all antibodies are structurally similar, with differences according to (1) the physiological class to which they belong, (2) the animal species, (3) individual Mendelian traits ("*allotypes*"), and (4) the exact sequence of the regions directly involved in antigen recognition. For the main part, the latter regions consist of three so-called "hypervariable" regions whose three-dimensional fit ensures a kind of structural complementarity to part of the antigen, responsible for its specific recognition. In addition, a strange and fascinating polymorphism has been discovered: *idiotypes*. Each individual reacts to a given antigen by producing a characteristic set of idiotypes. Different antibodies can have very different affinities for their antigen. The extreme diversity of antibodies (a mouse can make 10^9 different antibodies), which endows the individual with an enormous repertoire, is now largely understood in terms of structural rearrangements at the DNA and RNA level among the many genes coding for antibodies.⁵

A given antibody (Ab1), and, more specifically, a given idiootype, can be used itself as an antigen and elicits the synthesis of anti-antibody (Ab2), also called anti-idiootype, which, in

turn, can elicit the production of anti-anti-antibody (Ab3) or anti-anti-idiotypic, etc. Since Ab1 and Ab3 are both to some degree complementary to Ab2, Ab1 can be expected to resemble Ab3. The major importance of these interactions is beginning to be understood in terms of the "idiotypic network".^{4,6} The basic idea of the idiotypic network hypothesis is that the immune system, being complete (able to recognize the entire world of antigens), cannot avoid the recognition of itself. There is, then, a coexistence of idiotypes and anti-idiotypes within the repertoire of one individual (which can indeed be demonstrated experimentally). Therefore, the immune system has an "inner life" and displays regulatory pathways governed by idiotypic interactions. Using these ideas, it has been possible to manipulate the immune response in a way predetermined by the research worker. For example, anti-idiotypic antibodies can induce the production of antiviral or antibacterial antibodies in mice, rabbits, chimpanzees, etc., that have never encountered the microbe (idiotypic vaccines).

B. THE IMMUNE RESPONSE

When the immune system is brought into contact with a given antigen for the first time, the typical response is the production of a low level of antibody after a lag of several days (the "*primary response*"). If we now inject the same antigen again, the system can react more efficiently, producing *more* antibody after a *shorter* time lag and for a *longer period* (the "*secondary response*"). On the other hand, the immune system does not produce antibodies against antigens encountered early in the life of the organism. This important property, called *tolerance*, prevents the synthesis of antibodies against self-constituents. Presumably related to tolerance is the phenomenon of immune *paralysis*. Often, a first contact with a dose of antigen too low to elicit an efficient primary response will prevent any further response if more antigen is introduced (low-dose paralysis). If, on the other hand, the first antigen dose is too high, or if the organism is exposed repeatedly, the system may also become refractory to further contact with the antigen in question (high-dose paralysis).

Thus, in the absence of an antigen, the immune system can exist in several stable states, according to its past experience: a "*virgin*" state if it has never "*seen*" anything resembling the antigen in question, a state of *immune memory* if an earlier contact with the antigen prepared it to respond more efficiently, or a "*suppressed*" state if the previous contact with the antigen induced paralysis.

The immune response involves complex and only partially understood interactions among several large classes of lymphocytes, notably B lymphocytes, which ultimately secrete the antibodies, regulatory lymphocytes called T lymphocytes because they mature in the thymus, and their precursors. T lymphocytes are classically classified into T_h (helper) and T_s (suppressor) lymphocytes. Each of these classes comprises a large spectrum of cells as regards the specificity of the antibodies that will ultimately be produced.

In this chapter, for convenience we will focus on the small fraction of immune cells involved in the response to a given antigen and consider among this population "compartments" corresponding to the B, T_h , and T_s lymphocytes and their precursors. When we speak of cells "specific" to an antigen, we mean cells involved in subsequent production of antibodies directed against this particular antigen.

Antigens are usually not active as such; they are "presented" at the surface of appropriate cells (antigen-presenting cells), and it has become apparent recently⁷ that what is presented is, in fact, a choice of small peptides originating from the initial antigen molecules. Lymphocytes have surface receptors, some of which are antibodies (in B cells) or related molecules (in T cells). Contact between cells can be via antigen molecules or via idiotypic interactions between idiotypic and anti-idiotypic cell-surface receptors. In addition, certain lymphocytes emit extremely active molecules, such as lymphokines, which can take part in regulation without cell contact.

C. LYMPHOCYTE INTERACTIONS

These interactions are exceedingly complex and, in fact, far from completely understood. We will try to give a brief description of the principal interactions. It will necessarily be not only simplified, but also somewhat arbitrary: we have chosen those interactions that seemed to us the most relevant.

In the presence of an antigen, a population of helper T lymphocytes (T_h) specific to this antigen develops from the population of precursors. Once present, this population may persist in the absence of antigen. On the other hand, the T_h cells induce the development (from a pool of precursors) of a population of suppressor T lymphocytes (T_s), so called because they tend to suppress the development of the T_h population that induced it. Here, too, there is an autocatalytic component: the T_s population is induced by the T_h cells, but once present, it can persist independently of the T_h cells that induced it.

B lymphocytes, which will secrete the antibodies, are recruited from a pool of precursors by T_h lymphocytes of appropriate specificity, but the antigen, which has already served to induce the specific T_h population, is required again during B-cell activation and maturation.

Thus, the antigen exerts a double positive action (directly, and indirectly via T_h cells) on the stages of B-cell maturation. However, it also acts at a third level. As proposed by Lederberg⁸ and later confirmed,⁹ immature B cells are inactivated by the antigen corresponding to their specificity. This accounts for tolerance; immature B cells specific to a protein of the organism itself will meet this protein before reaching the next stage of development, and be inactivated.

II. FORMAL DESCRIPTION OF ASPECTS OF THE IMMUNE RESPONSE

A. GRAPH OF INTERACTIONS

It must be borne in mind that the description just given represents a selection of those interactions that seemed to us most significant, from among a tremendous mass of experimental facts. This description can be represented by the graph of interactions shown in Figures 1 and 2.

The "core" can be considered as an entity that sends information toward the other elements of the system, but is itself influenced only by the antigen. Thus, provisionally treating the antigen as an input variable, we will first analyze this part of the model separately. In fact, in addition to the feedback loops presented in the core of the model, there is an additional negative loop involving the antigen: the antibody, once produced, will neutralize the antigen, thus impairing its further inducing effect. This is why we can only *provisionally* treat the antigen as an input variable, switched on whenever we add antigen and off soon after antibody appears.

The schemes presented in Figures 1 and 2, like the verbal description (Section I.C), lack information about the connections between interactions. For example, the further development of compartment T_h depends on its own present state, on the state of compartment T_s , and on antigen. But how are these influences connected?

Whatever the formalism used — naïve logical, generalized logical, or differential — we will have to specify these points, i.e., make additional assumptions. As we will see, our work combines all three approaches. The naïve logical description was quite useful in the elaboration of the model. At the other extreme, the differential description permitted us to treat the role of antigen in a more refined way.

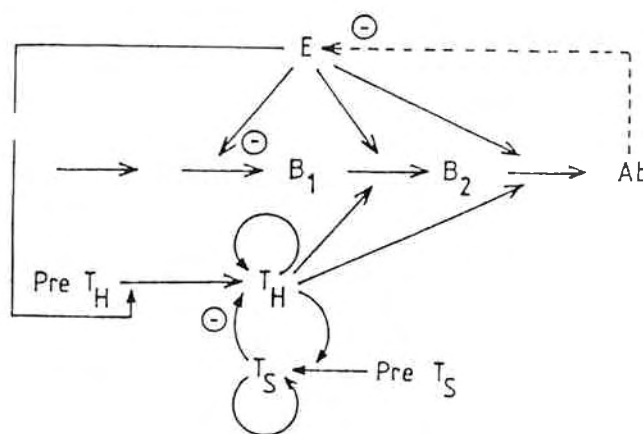


FIGURE 1. Schematic diagram of the interactions. E, antigenic determinant or epitope; Ab, antibody; B₁, virgin B cells; B₂, more mature B cells; T_H, T helper cells; T_S, T suppressor cells. Unless otherwise specified, the interactions are positive. Clearly, the core of the graph is the multiple-loop interaction between the T_H and T_S, both of which display an autocatalytic component (Figure 2).

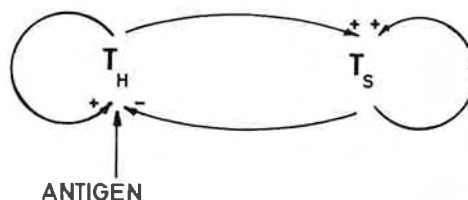


FIGURE 2. The core of the model.

B. NAIVE LOGICAL DESCRIPTION

Let us first define the variables and functions used: (1) h, H : the variable and function associated with the T_H cells, (2) s, S : the variable and function associated with the T_S cells, and (3) e : antigen. Although we previously¹ used a three-level variable (even before the development of our generalized logic), we will first treat e as a binary variable. The interactions between h, s , and e , shown in Figure 2, can be connected in several ways, two of which were selected by Kaufman:²

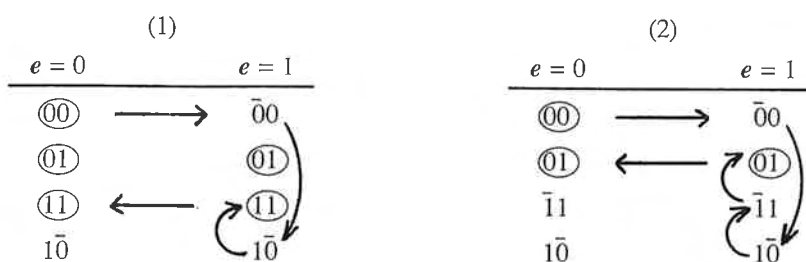
$$\begin{cases} H = e \cdot \bar{s} + h \\ S = h + s \end{cases} \quad (1)$$

$$\begin{cases} H = (e + h) \bar{s} \\ S = h + s \end{cases} \quad (2)$$

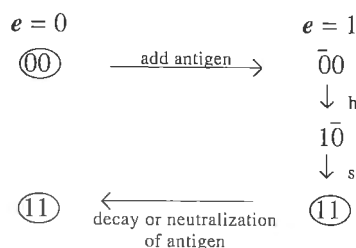
Concretely, Equations (1) say that T_H cells are recruited from a (nonformalized) precursor pool if the antigen is present and there are no T_S cells. Once "primed" in this way, the T_H

compartment will persist autocatalytically. Equations 2 differ from Equations (1) in that not only the establishment mechanism, but also the maintenance require the absence of suppressor cells. The compact state tables are

TABLE 1



In the absence of antigen ($e = 0$), Model 1 has three stable states, 00, 01, and 11, respectively characterized by the absence of both T_h and T_s , the presence of T_s alone, and the presence of both. These stable states nicely account for the observed "virgin", "suppressed", and "memory" states. The first is found in naive animals that have not been exposed to the antigen in question, the second in animals that are blocked in a state of immune paralysis, and the third in animals that have already been exposed to antigen and have kept the memory of this contact in spite of the disappearance of the antigen. According to this model, one can proceed from the "virgin" state 00 to the memory state 11 as follows:



This is due to the fact that state 00, which was stable (00) in the absence of antigen, is no longer stable (00) in its presence. Indeed the whole point of the immune system is to respond to the presence of antigen. Thus, once antigen has been added (shift from left to right column), the system follows the pathway $00 \rightarrow 10 \rightarrow 11$. Since state 11 is stable (11) irrespective of the presence or absence of antigen, the system will remain in state 11 even after antigen has decayed or been neutralized by antibody. In the analysis of the complete system, studied in parallel by logical and differential methods,^{1,2} it can be seen that a system in the "memory" state reacts faster and more efficiently than a system in the "virgin" state. This accounts for the well-known difference between the primary and secondary responses. Furthermore, if the system is in the "suppressed" state, the model accounts for the paralysis; adding antigen does not remove the system from state 01, in which B cells cannot mature for lack of T_h cells. There is, however, an unpleasant point in this analysis: the suppressed state 01 cannot be reached from the virgin state 00. We will see in the next section that this difficulty disappears in the generalized logical description of the same model.

Let us now briefly look at Model 2, just to notice that the naïve version of this model has only two stable states in the absence of antigen, $\textcircled{00}$ and $\textcircled{01}$, and cannot account for the state of immune memory. At this point, we would be tempted to reject the model. However, we will see that the generalized logical version is more satisfactory.

C. GENERALIZED DESCRIPTION

Since in Systems 1 and 2, the variables h and s both act at two places, we should consider two thresholds, and thus three logical values, for each of them. We associate a weight K with each term of our logical expressions. For the term $e \cdot \bar{s}$, it was found convenient³ to include the antigen concentration e in the constant K_{12} . Thus, at very low concentration of antigen, the discretized value K_{12} will be 0, and at higher concentrations, it will be 1 or 2. The generalized system corresponding to the naïve relation 1 is

$$H = d_h(K_{11}h + K_{12}\bar{s})$$

$$S = d_s(K_{21}h + K_{22}s)$$

with the level of antigen included in K_{12} .

Since both h and s have two thresholds, we have four possible subsystems, according to the relative values of the thresholds. We consider here the situation in which $\theta_{12} > \theta_{22}$ and $\theta_{11} > \theta_{21}$, and the relations become:

$$\begin{aligned} H &= d_h(K_{11}^2h + K_{12}^2\bar{s}) \\ S &= d_s(K_{21}^1h + K_{22}^1s) \end{aligned} \quad (3)$$

TABLE 2
State Tables of Generalized Model 1

h	s	H	S
0	0	K_{12}	0
0	1	K_{12}	K_{22}
0	2	0	K_{22}
1	0	K_{12}	K_{21}
1	1	K_{12}	$K_{21} + 22$
1	2	0	$K_{21} + 22$
2	0	$K_{11} + 12$	K_{21}
2	1	$K_{11} + 12$	$K_{21} + 22$
2	2	K_{11}	$K_{21} + 22$

s	0	1	2
2	02/0 K_{22}	12/0 $K_{21} + 22$	22/ $K_{11}K_{21} + 22$
1	01/ $K_{12}K_{22}$	11/ $K_{12}K_{21} + 22$	21/ $K_{11} + 12K_{21} + 22$
0	00/ $K_{12}0$	10/ $K_{12}K_{21}$	20/ $K_{11} + 12K_{21}$
	0	1	2
	h		

Note: Model 1 based on Relation 3.

In Table 2, the general state table is presented in two versions. In Figure 3, we have adopted the logical parameters $K_{11} = 2$, $K_{21} = 1$, and $K_{22} = 2$. The value of K_{12} is 0 for no

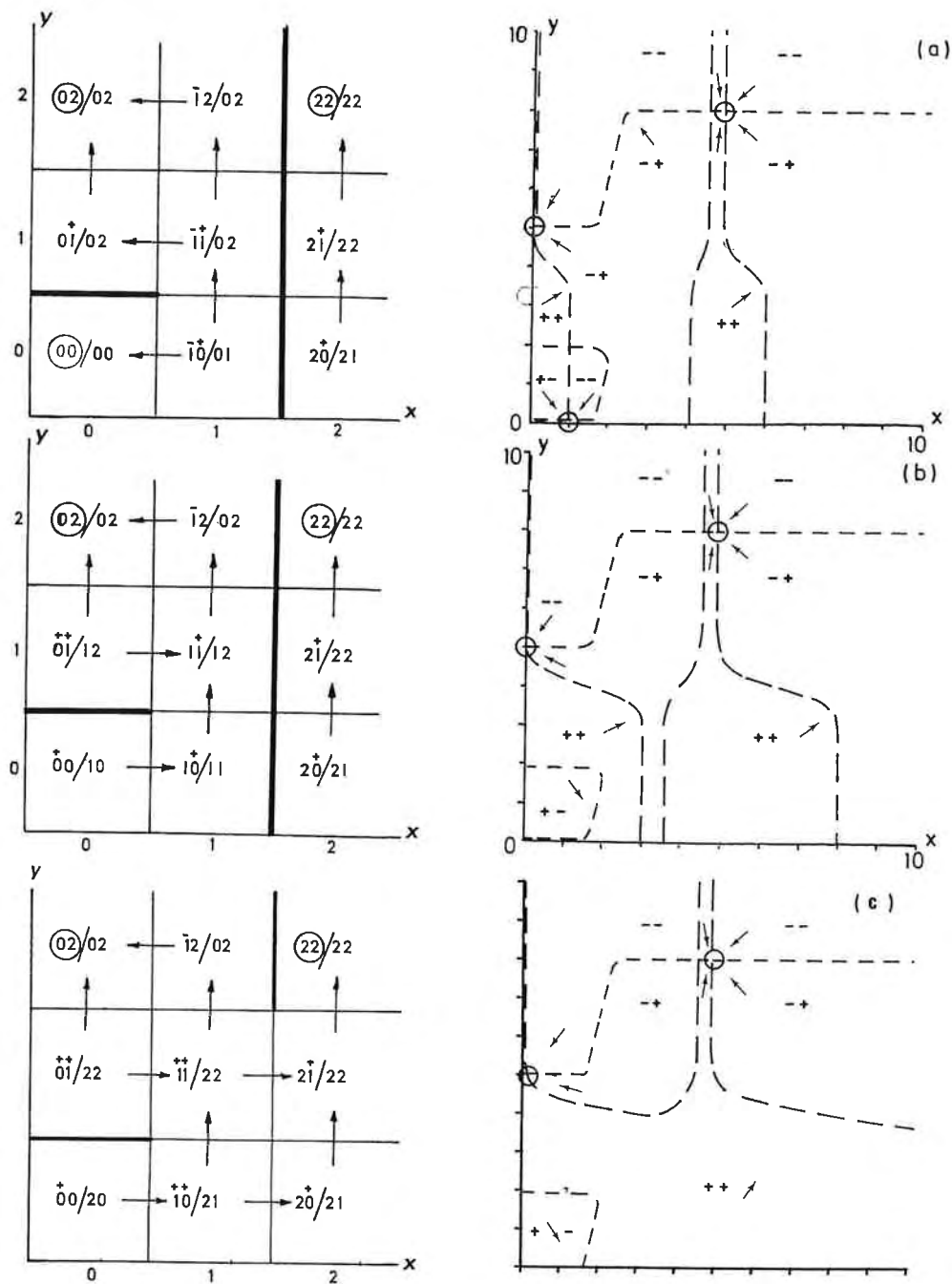
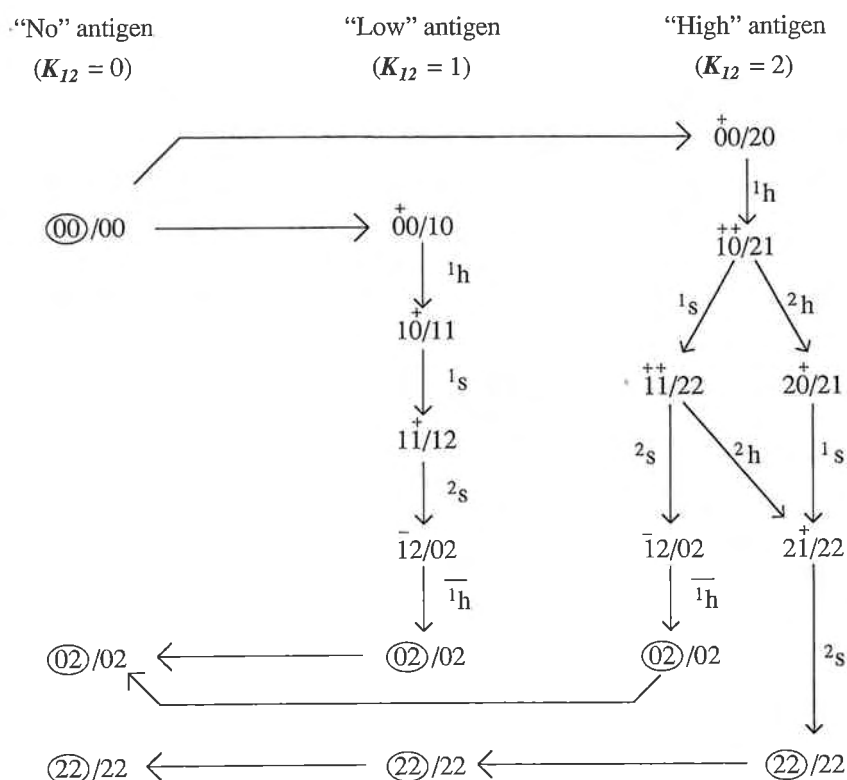


FIGURE 3. State table of system (3) (left) and nullclines of the corresponding differential system (right) for three values (0, 1, 2) of the logical parameter K_{12} , corresponding to low, middle, and high antigen concentration, respectively. The other logical parameters are $K_{11} = 2$, $K_{21} = 1$, and $K_{22} = 2$. The parameters used in the differential description have been chosen within the domain of agreement with the logical parameters. As in other chapters, the nullcline $dx/dt = 0$ is symbolized by long dashes (— — —) and dy/dt by short dashes (- - - -). The Hill number used is $n = 20$. In this figure, variables h and s are labeled x and y , respectively.

TABLE 3
A Combination of the Three State Tables of Figure 3



Note: Each of the three columns corresponds to a value of K_{12} ("no", "low", and "high" antigen).

(or very low) antigen, 1 for low antigen, and 2 for high antigen. For each case, we see the state tables (left) and nullclines of the differential description (right).

Let us start from the virgin state $00/00$ in the absence of antigen (Table 3, a) and add a little antigen (shift from grid a to grid b). State 00 is no longer stable ($00/10$) and the system will proceed via three intermediate states to the suppressed state $02/02$. After exhaustion of the antigen (slow because in this suppressed state no antibody is produced), the system returns to grid a, in which state 02 is stable, and thus remains in the suppressed stable state $02/02$. We can now add either low (shift to grid b) or high antigen (shift to grid a) without any result; the system will remain in the suppressed state. This accounts very well for low-dose paralysis; after being injected with a too low dose of antigen, the animal becomes refractory to immunization, even with a high antigen dose.

Starting again from the virgin state $00/00$ in the absence of antigen (left part of Table 3a), let us give a higher dose of antigen (shift to the right part of Table 3). Again, state 00 is no longer stable ($00/02$), followed by $10/21$, and, according to the time delays, the system will move to stable state $02/02$ or $22/22$. In the second case, antibody will be synthesized (as one can see in the treatment of the complete system). The antigen will thus be rapidly neutralized and the system shifts back to grid a of Table 3a. Since state 22 is stable in the

absence as well as in the presence of antigen, the system remains in the "memory" state $\textcircled{22}/22$. From now on, a second addition of antigen will result in an accelerated antibody production (because we are already in state $\textcircled{22}/22$, with T_h cells of the proper specificity present and ready to induce B cells to synthesize antibody).

In the naïve description of this system, there was a problem because there was no pathway from the virgin state to the suppressed state. We have seen above that in the generalized description there is no such problem. This is not a discrepancy between the naïve and generalized descriptions. We must remember that our naïve description is a particular case of the generalized one, in which $\theta_{11} = \theta_{21}$, $\theta_{12} = \theta_{22}$, and all logical parameters are 1. We see now that there are logical parameter values for which the description fits well with experimental solutions.

As briefly mentioned above, Model 2, which seemed unsatisfactory in its naïve version, becomes acceptable in the generalized description. More specifically, in the naïve description there are only two stable states in the absence of antigen (no "memory" state $\textcircled{11}$). As shown in Figure 4, according to the parameter values, there may be three stable states ($\textcircled{00}$, $\textcircled{01}$, $\textcircled{21}$) or two stable states ($\textcircled{00}$, $\textcircled{01}$) and a cycle that suggests an oscillation of h and s , indeed observed in some conditions.

D. DIFFERENTIAL DESCRIPTION

The discrete approach has been of great help, if only because its simplicity permitted us to check a number of possible variants of the connections between variables and select the most appropriate ones. At a later stage, we selected the most interesting sets of values of the logical parameters and injected their real counterparts into the homologous differential equations. The fit of the generalized logical description with the differential description is illustrated in Figures 3 and 4, which show in parallel the logical state tables and the nullclines of the differential description. In these figures, we have used nullclines corresponding to differential equations with rather steep interactions ($n = 20$). It may be argued that the fit of the logical description with a differential description using highly nonlinear interactions is not surprising. In fact, the qualitative fit persisted when we used differential systems with part of the interactions linear and part sigmoid with $n = 2$ (Reference 2) — in full agreement with the earlier observations of Glass and Kauffman.¹⁰ In the logical description, we found it convenient although by no means indispensable to provisionally treat antigen as if it were an input variable. There is no reason to adopt this attitude for the differential description. A description of the complete system is given in detail in Reference 2.

Like the nervous system, the immune system is an exceedingly complex network, and any attempt to formalize it in detail would be perfectly hopeless. Here, we have reasoned in terms of the small fraction of the network that recognizes a given antigen and considered a small number of cell types, treating collectively all the cells belonging to one of these categories. In addition, we have considered only a small number of interactions that seemed to us especially relevant.

It is gratifying to find that in spite of such drastic simplifications, one can get a reasonably correct view of several important aspects of the immune response. The next step should consist of describing and analyzing specific aspects of the immune response more accurately. As in other fields, we expect an efficient feedback involving the predictions of the models, experiments inspired by these predictions, and appropriate modifications of the models.

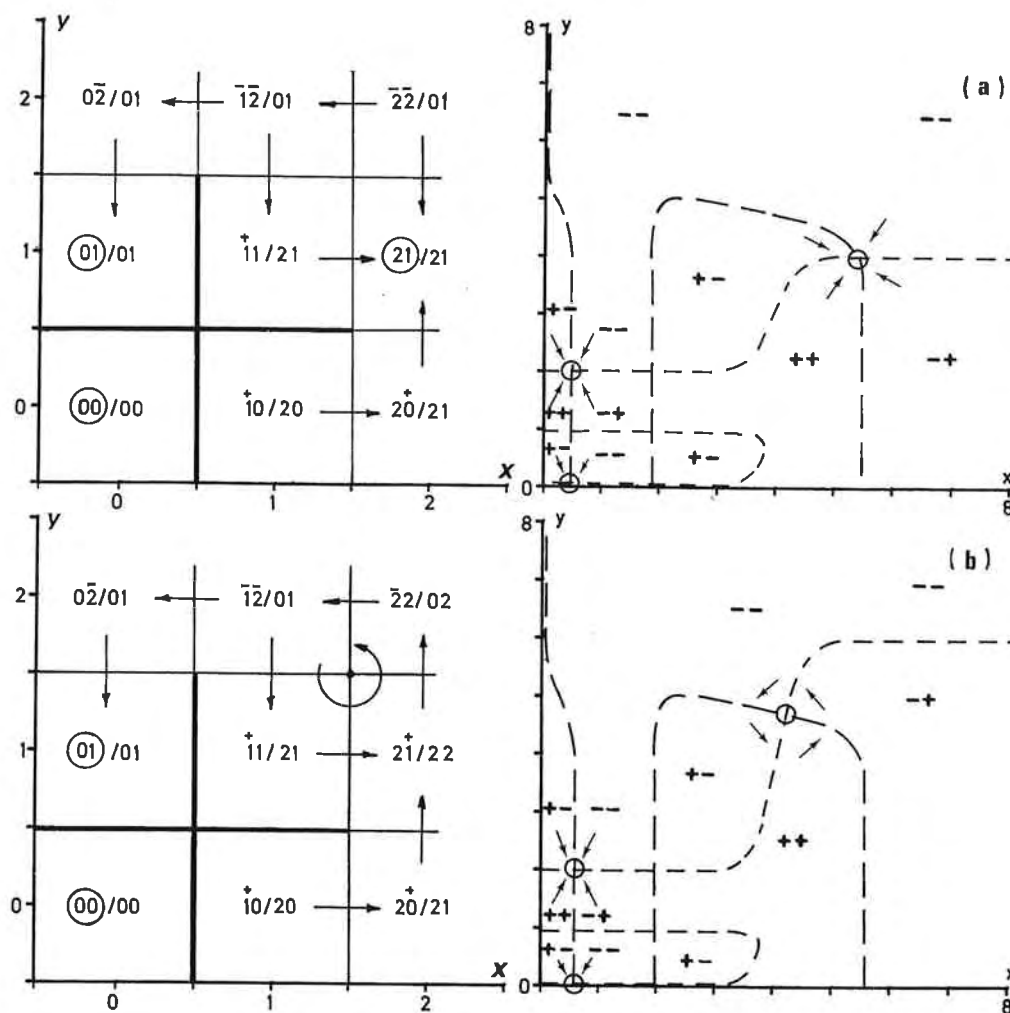


FIGURE 4. Generalized logical (left) and differential (right) description of the system:

$$H = d_h [{}^2s (K_{11} {}^1h + K_{12})]$$

$$S = d_s (K_{21} {}^2h + K_{22} {}^1s)$$

whose naïve logical description is (2), in the absence of antigen. Here, $K_{12} = 0$, K_{21} and $K_{22} = 1$, and $K_{11+12} = 2$. The difference between (a) and (b) is that in (a), $K_{21+22} = 1$, and in (b), $K_{21+22} = 2$. As a result, the logical stable state 21/21 (a stable node in the differential description) is replaced by a cycle (a stable focus in the differential description). Also see the legend to Figure 3.

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Chapter 23

ON DETERMINATION AND DIFFERENTIATION

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I. DIFFERENTIATION SEEN BY A POSITIVE FEEDBACK LOOP FANATIC

It has become clear that *some* transmissible differences among cell lines of a population are due to differences in the structure of the genetic material. To choose examples at very different levels in the hierarchy of living organisms: yeast cells of opposite mating type differ by a well-defined DNA transposition event, and various cell lines in the immune system of vertebrates differ by DNA rearrangements that bring together pieces of DNA that were initially separated (typically, the variable and constant parts of genes coding for immunoglobulins).

However, it seems more and more likely that the vast majority of the differences among cell lines of a given organism are *not* due to genetic alterations; most of our cells are probably genetically identical. All of our genes are present in all cells, but in each cell type only certain genes are expressed at a significant rate.

The idea that slight environmental differences can explain why a gene is on in one cell type and off in another is in many cases untenable because the differences persist when the two cell types are grown in identical environments in cell culture. Thus, in many cases two genetically identical cells in identical conditions behave in heritably different ways, presumably reflecting different events that took place in their past history. These are typical *epigenetic differences*, alluded to in Chapter 17.

To account for the fact that, say, a hepatocyte produces serum albumin whereas a fibroblast does not, it is often said that this is either because the latter contains a repressor that is absent in the former or because the former contains an activator absent in the latter. Although either (or both!) of these hypotheses may be true, two objections can be raised to this type of "explanation". First, assuming that one of these hypotheses is correct, it merely displaces the problem. The question now becomes, for example, "why does the fibroblast produce a repressor, whereas the hepatocyte does not?" Of course, we do not mean to imply any criticism of this type of *approach*. Indeed, progress is often via small steps that gradually deepen the experimenter's understanding of the situation. Our point is simply that such a finding would shed no light on the *origin* of different cell types.

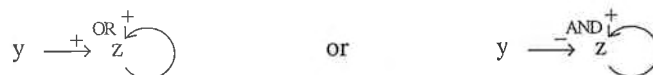
The second objection is perhaps more serious. In fact, what is remarkable is not that a given cell type produces this or that protein at a particular moment, but, rather, that the system behaves as though at some point during its development a decision was made and (after a lag) the cell began to produce certain proteins and from that point on continued producing them heritably, irrespective of the environmental conditions.

As we shall see, both objections vanish if we assume that **each binary choice** (between producing a given protein or not, between producing either protein a or protein b, etc.) is **directed by a positive feedback loop**, i.e., by an autocatalytic process (direct or indirect). The first objection disappears because the cause of a specific phenotype no longer forms a long regression (of the sort "a is present because b is present, and b is present because c is absent, and c is absent because. . ."), but comes back to itself ("a is present because . . . a is already present") in a positive feedback loop. The second objection disappears because, as we have seen, positive loops permit a choice that, once made, is stable in the absence of a major external change.

Note that "epigenetic" should by no means be opposed to "genetic". In order to be able to exist in two or more stable states, a system must have a logical structure with the appropriate positive loops, and this is partly ensured by proper interactions between genes and regulators.

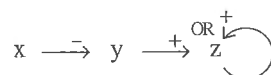
On the other hand, assuming that the possibility to have such choices is due to the presence of positive loops in the genetic network, how are decisions made? We have seen that

although a simple one-element positive loop provides two stable states, it operates as a vicious circle and cannot by itself make the decision. However, in simple devices like those illustrated by the graphs



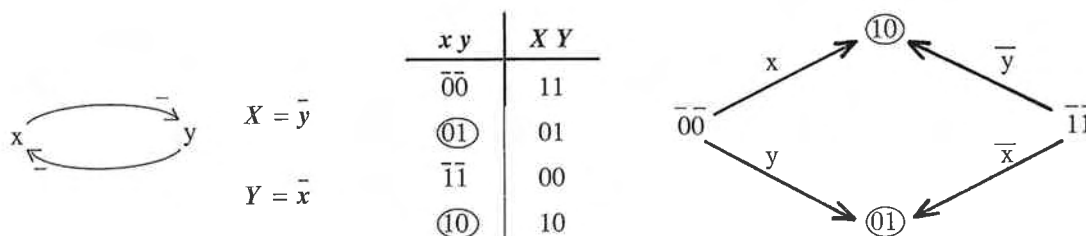
y can serve as a trigger (or push button). In the first system, if gene Z is initially off, a transient pulse of y will switch it on permanently, and in the second system, if gene Z is initially on, a transient pulse of y will switch it off permanently.

We can now ask, What decides whether (and when) the push button will act? One simple device, inspired from phage λ , was presented in Chapter 14:



Here, starting from a “virgin” state (all products absent), the decision whether or not to turn z on basically depends on the relative accumulation rates of x and y , as discussed in Chapter 14.

Another elementary decision-making device is the simple two-element positive loop:



From the initial state $\bar{0}\bar{0}$, the system will go to (and stabilize at) $\bar{1}0$ or $\bar{0}1$, according to whether $t_x < t_y$ or $t_y < t_x$; and from $\bar{1}\bar{1}$, it will go to (and stabilize at) $\bar{1}0$ or $\bar{0}1$, according to whether $t_{\bar{y}} < t_{\bar{x}}$ or $t_{\bar{x}} < t_{\bar{y}}$.

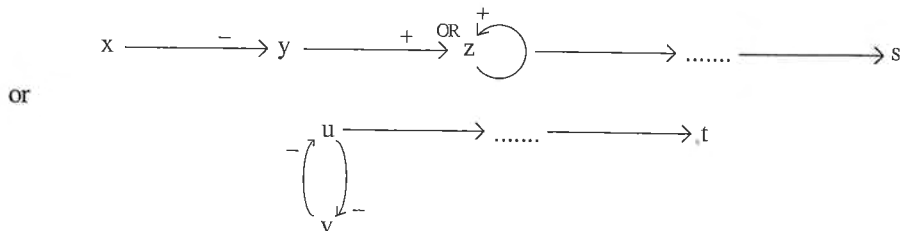
Of course, for both devices, if all time delays were absolutely constant, the pathway would be irrevocably fixed. With the second device, for example, if $t_x < t_y$ and $t_{\bar{x}} < t_{\bar{y}}$, the system would invariably proceed from $\bar{0}\bar{0}$ to $\bar{1}0$ and from $\bar{1}\bar{1}$ to $\bar{0}1$. However, even in a homogeneous cell population, time delays are unlikely to be identical in all cells: rather, they would be expected to have a distribution around a mean value. Thus, if the mean value of t_x , say, is smaller than that of t_y , most of the cells will follow the corresponding pathway $\bar{0}\bar{0} \rightarrow \bar{1}0$, but, depending on the overlap of the distributions, a certain fraction of the cells will follow the minority pathway ($\bar{0}\bar{0} \rightarrow \bar{0}1$). Using this principle, we have simulated the choices governing the decision by bacteriophage λ to integrate or not into the host genome after infection.¹

In the above discussion, we treated the decision of cells to choose one or another pathway as if it were a stochastic process. Although this may be correct in some cases, it is certainly not generally true. It seems more likely that variations in the value of certain time delays are, in fact, determined by differences in the local environment. It has long been known, for example, that many egg cells are anisotropic and can be described in terms of “morphogenetic gradients”, i.e., concentration gradients of substances involved in morphogenesis. We

would be tempted to postulate that certain delays are affected by these substances, making the choice of developmental pathway depend on the precise position in the gradient.

Embryologists distinguish between *determination* and *differentiation*. Determination is the decision itself, the commitment to follow one or another pathway. However, two cells already committed to different fates often remain indistinguishable for some time, both morphologically and with respect to the proteins they synthesize. Only later, when their specific potentialities are expressed, can the cells be considered differentiated.

We are thus interested in the possible mechanisms of (1) the decision itself (determination), (2) its maintenance, and (3) its final expression (differentiation). We will briefly discuss these three points in the context of devices like:



in which x , y , z , u , and v are regulators and s and t are proteins characteristic of specific differentiation pathways ("s" or "t" differentiation).

(1) Product y clearly acts as a push button and is thus involved in the decision. However, even in the simple model proposed here, it is not the only decision-making element; x is also involved, as discussed in Chapters 4 and 14.

In the second scheme, rather than being either "on" or "off", the positive loop is in either the "u" or the "v" position, according to the relative values of the time delays (t_u vs. t_v if the initial state is $\bar{0}\bar{0}$, t_u vs. t_v if it is $\bar{1}\bar{1}$).

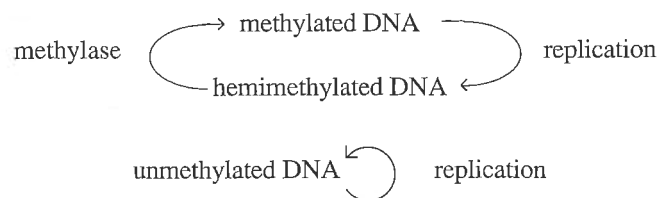
(2) The *maintenance* of the determination from the committing decision to the actual differentiation consists of the maintenance of the positive loop in whichever state the decision set it. For the first scheme, if z exerts a positive effect on the synthesis of s (direct or indirect), the loop must be maintained in the "on" position, with continued synthesis of z . If on the contrary, z exerts a negative effect on s , the "s" differentiation requires that the loop remain "off", with continued lack of synthesis of z . In the second scheme, the choice is between the presence of u or the presence of v ; either of these regulators could exert a positive or negative effect on the synthesis of t . Again, the maintenance of the decision simply requires maintaining the loop in the appropriate position.

(3) As mentioned above, there is often a long lag between the decision (determination) and its execution (differentiation proper). There are presumably different causes, according to the case. While a cascade of positive controls would hardly justify long delays, a simple possibility would be that setting the positive loop results in switching off a negative control gene whose product has a long life span. In this case, the effect would be delayed until the negative regulator has decayed.⁹

II. CIS VS. TRANS PROCESSES

Virtually all naturally occurring DNA contains a small number of methylated bases, formed by specific DNA methylases. In certain cases, this methylation plays an interesting role in gene expression. Each methylating enzyme recognizes a specific DNA sequence that is often symmetric (i.e., the two strands of the double helix have the same sequence running in opposite directions), and the methylation takes place at a specific base on each strand.

Semiconservative DNA replication first produces hemimethylated DNA, and some methylases can use only this as a substrate, with essentially no activity on completely unmethylated DNA. For these enzymes, the methylation process is thus autocatalytic: only DNA produced by replication of methylated DNA can be methylated.



This process is operative in bacteria as a protection against the restriction endonucleases they produce, which recognize the same sequence as the corresponding methylases and cut the DNA unless the sequence is methylated. This permits the cell to distinguish between its own DNA, which is always methylated (or hemimethylated) at the appropriate sites and therefore substrate for the methylase but not for the endonuclease, and foreign DNA, normally unmethylated at these sites and so substrate for the endonuclease but not the methylase. Thus, foreign DNA injected, for example, by a phage will usually be destroyed.

Certain genes are silent or expressed according to whether or not specific DNA sequences are methylated². This situation seems to be particularly exploited by higher organisms. For example, in some species in which females have two X chromosomes but males only one, female cells generally express only one of the X chromosomes, the other being kept silent concomitantly with self-propagating methylation. In theory, at least, this type of process could be used to turn on a whole block of genes in response to a signal such as temporary inhibition of the methylase, resulting in permanent demethylation of the genes in question.

Should this process be considered genetic or epigenetic? What are the criteria? Normally, we think of epigenetic processes as being mediated by products — activators or repressors, for example — that affect DNA expression, whereas genetic processes alter the DNA itself. DNA methylation straddles this distinction: the DNA is chemically altered, but the base sequence is preserved. The fact that the transmissibility of methylation can be accounted for by a positive feedback loop is not an argument to classify it as epigenetic. After all, genetic changes also behave autocatalytically and can thus be described by a positive loop. A better reason to consider it epigenetic is that it is not transmitted through sexual reproduction. On the other hand, there is one essential feature of methylation that distinguishes it from other epigenetic differences.

Suppose that we fuse two cells, one in which the loop $x \overset{+}{\curvearrowright}$ is on (\underline{x} present, gene \underline{X}

active), the other with the loop $x \overset{+}{\curvearrowright}$ off (\underline{x} absent, gene \underline{X} silent). In the hybrids, \underline{x} is present, so not only will the active copies of gene \underline{X} remain active, but the silent copies will be switched on. The product \underline{x} produced by the first genome acts “in trans” to switch on the \underline{X} gene of the second genome. Consider now what would happen if we fuse two cells, one in which gene \underline{Y} is unmethylated and active, the other with gene \underline{Y} methylated and inactive. Here, the difference in activity of the two copies of gene \underline{Y} does not result from the presence or absence of a cytoplasmic factor; the DNA methylase is present in both cells. The difference resides in the DNA itself, whose state, methylated or unmethylated, is self-propagating. We thus expect each copy of gene \underline{Y} in the hybrid to remain in the same state it was in,

active or inactive, before the cells were fused. In this case, there is no *trans*-acting factor; the determinant of the state (i.e., the methylation state of the DNA) acts only *cis*.

III. CELL HYBRIDS³

Consider a line of fibroblasts that do not synthesize serum albumin and a line of hepatic cells that do synthesize it. As discussed above, we would suggest that the permanent maintenance of the albumin gene in the on or off state is determined by the state of a positive loop.

Suppose it depends on a one-element loop $x \overset{+}{\curvearrowright}$. We can account for the two states of the

albumin gene either by a circuit of the type $x \overset{+}{\curvearrowright} x \longrightarrow \neg a$ (negative regulation), with

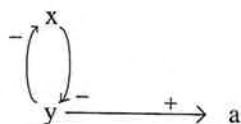
the loop on in the fibroblasts and off in the hepatocytes, or by $x \overset{+}{\curvearrowright} x \longrightarrow +a$ (positive

regulation), with the loop off in the fibroblasts and on in the hepatocytes. What would happen if we fused a fibroblast with a hepatocyte? According to the first hypothesis, the repressor x would be present in the hybrid cell, thus ensuring its continued synthesis and the extinction of the hepatocyte albumin gene. In the second hypothesis, the activator x would be present in the hybrid cells, thus ensuring its continued synthesis and the expression of the albumin genes. (We assume that the twofold dilution of x due to cell fusion does not lead to a subthreshold concentration.) The experimental observation is that the vast majority of hybrid cells do not produce serum albumin^{4,5}. If the control is indeed via a one-element positive loop, we would conclude that this element is a negative regulator of the albumin gene. The opposite situation is encountered for the third factor of complement, which continues to be synthesized in hybrid cells.⁴ If a one-element loop is involved, this would suggest that expression is positively controlled by the regulator.

This situation would be similar if the circuit involved a two-element positive loop of the

type $x \overset{+}{\curvearrowright} y \overset{+}{\curvearrowright} x$, in which the two elements x and y are either both present or both

absent. However, for a loop of the type $x \overset{-}{\curvearrowright} y \overset{-}{\curvearrowright} x$, the two steady states are "only x present" and "only y present", and one could assume either that x inhibits albumin synthesis or that y activates it. Assuming the latter, we have the following graph of interactions, logical relations, and state table:



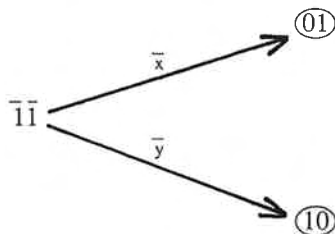
$$X = \bar{y}$$

$$Y = \bar{x}$$

$$A = y$$

xy	XY
$\bar{0}\bar{0}$	11
$\textcircled{01}$	01
$\bar{1}\bar{1}$	00
$\textcircled{10}$	10

In this model, the loop is in state \underline{x} ($\textcircled{10}$) in fibroblasts (no production of albumin) and in state \underline{y} ($\textcircled{01}$) in hepatocytes (production of albumin). Now what do we expect a hybrid cell to do? Initially, it will contain both \underline{x} and \underline{y} (again, we assume the twofold dilution can be neglected), so the synthesis of \underline{x} and \underline{y} will be turned off and the two products will decay:



Since the albumin gene is generally turned off in the hybrids, our model should stipulate that \underline{x} decays more slowly than \underline{y} , resulting in the transition $\bar{1}\bar{1} \xrightarrow{\bar{y}} \textcircled{10}$. For a gene that continues to be expressed in the hybrid cells, it would be sufficient to postulate that $t_{\bar{x}} < t_{\bar{y}}$, without requiring a different regulatory mechanism. In fact, when fibroblasts are fused with hepatocytes containing a double chromosome complement, albumin synthesis is no longer extinguished⁶, as if the increased concentration of regulator \underline{y} increased its decay time sufficiently to make $t_{\bar{y}}$ greater than $t_{\bar{x}}$.

In summary, the idea that the on or off state of genes in different cell lines is commanded by the state of a positive loop can account for a number of observations. If the loop has only a single element, one must assume different control mechanisms for cases of extinction and activation in hybrid lines, negative and positive, respectively (assuming the twofold dilution is negligible). In contrast, if the positive loop includes negative interactions, like the loop

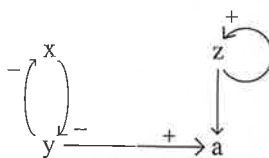


\underline{x} and \underline{y} , both extinction and activation can be accounted for by a single model (in which the regulator can act positively or negatively) simply by postulating the appropriate off delays.

Our reasoning has been in terms of *trans*-acting regulators (cf. Section II above). In cases of extinction, like our example of serum albumin, this is clearly justified. For the third component of complement, which continues to be synthesized in hybrid lines, *trans* action has been shown by fusing two cell lines of different origin (mouse-rat, mouse-man, etc.). The resulting hybrids produce the third component specific to both species, showing that the fibroblast genes (normally silent) are indeed turned on.

However, it has become clear that not all experimental observations can be accounted for in terms of diffusible gene products only. Cases are known, for example, in which a gene that is turned off in the hybrid line can be reactivated by loss of specific chromosomes. Tyrosin aminotransferase (TAT) synthesis can be turned off in rat hepatoma cells by the introduction of a mouse chromosome 11 from a fibroblast, and elimination of this chromosome produces lines in which TAT is reexpressed. In these experiments, it is as though a negative regulatory gene on mouse (fibroblast) chromosome 11 could be expressed without turning on the homologous rat (hepatoma) gene. This is a *cis* effect: each copy of the negative regulatory gene follows its own pattern of expression. As discussed in Section II, self-propagating DNA methylation can account for this type of behavior.

The situation just described can be accounted for by models of the type:



in which the positive loop on z represents the autocatalytic character of DNA methylation; only unmethylated copies of gene \underline{z} will be expressed. A fibroblast has gene \underline{z} unmethylated (on) and is in the \underline{x} state, whereas a hepatocyte has gene \underline{z} methylated (off) and is in the \underline{y}

state. If these cells are fused, the loop $\underline{x} \rightleftharpoons \underline{y}$ will be adjusted since it depends on diffusible factors, but the \underline{z} genes will continue to behave as if nothing had happened. Thus, \underline{z} will be present in the hybrid cell and TAT will not be made. If we now select for loss of the fibroblast \underline{z} gene (presumably on mouse chromosome 11), the repressor \underline{z} will no longer be synthesized and, if the loop is in the \underline{y} state, the hybrid will turn on TAT synthesis.

One may ask whether *both* types of mechanisms, *cis* and *trans*, have to be invoked. Let us simply say that the occurrence of *trans* mechanisms is clear in some cases and of *cis* mechanisms in others, but it is not established whether both are involved in the control of a given protein.

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CONCLUDING REMARKS**TABLE OF CONTENTS**

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I. INTRODUCTION

Some of the ideas developed in this book are new; many are not. Our hope is to have reached a view of regulatory processes in which new and older ideas are organized in a coherent way. On the one hand, this view is perhaps too simple in the sense that we have emphasized what seemed to us really basic principles. On the other hand, the development of an appropriate formal description opens the way for an integrated approach to more complex regulatory networks. These concluding remarks concern (1) the regulatory process, (2) its description and analysis, and (3) some perspectives.

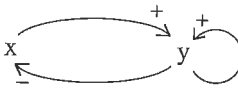
II. THE REGULATORY PROCESS

Regulation allows systems to take account of the internal as well as the external situation. The internal situation is taken into account via feedback interactions, that is, via closed chains of interactions (here called loops), which inform the organ that produces an element about the state of this element.

We have developed the idea that there are two fundamental types of regulations, homeostatic and epigenetic. Homeostatic regulation drives the elements of a system to or near a level intermediate between, and significantly different from, the basal ("off") and the maximal ("on") levels. Once there, the level is maintained at, or oscillates around, this supposedly optimal value. According to the case, we have a stable or an unstable focus. In contrast, epigenetic (or differentiative) regulation forces the elements of the system to choose between two extreme levels. Once reached, either level is maintained stably in the absence of major perturbations. Multistationarity is the property of systems whose structure allows for several steady states. Some of these steady states are attractors, each of which has its domain. Once the system has penetrated into the domain of an attractor, it will remain there in the absence of an external perturbation.

As discussed at length, homeostatic and epigenetic regulations are brought about by negative and positive feedback loops, respectively. These two types of regulation are in no way mutually exclusive. In complex networks, positive and negative feedback loops are often interconnected. They tend to keep their typical properties. An appropriate coupling of the two will generate both multistationarity and homeostasis, giving the system a choice among attractors, some of which may be oscillating. For example, in product activation (cf. Chapter

12), the molecular mechanism is described by $x \xrightarrow{+} y : \underline{x}$ is converted into \underline{y} , which catalyzes the conversion. In this reaction scheme, one sees only the positive loop of \underline{y} on itself. However, since \underline{x} exerts a positive effect on \underline{y} and \underline{y} a negative effect on \underline{x} , the

logical graph of interactions is , which includes the negative loop required for sustained oscillations.

As pointed out already, "epigenetic" should not be opposed to "genetic"; a genetic system will have multiple steady states only if the gene interactions comprise the appropriate positive loops.

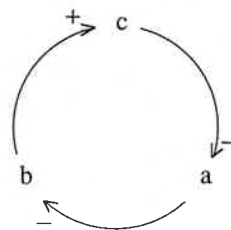
The appearance of *new* positive loops in the genome is a process that should be considered on the time scale of evolution, not of the individual. The situation, however, might be much more flexible in other systems, such as the nervous system. In higher organisms, many neurons interconnect at random, thus producing loops — each of which is necessarily negative or positive — and linear chains that can be connected to loops and operate under

their control. If one of these loops happens to be used, the connections will be reinforced and the loop may be stabilized. When such a loop is positive, it typically has two positions, say "on" and "off". Once structurally stabilized, it may persist for long periods in the "off" position. However, a proper signal can switch it on, thus revealing the potential behavior linked to the loop and its associated chains of neurons. To the extent that such a state of the system may correspond to a "mental image",¹ these processes could be involved in memory, and to the extent that the structural fixation of a loop depends on exercise, these processes could be involved in learning.

III. DESCRIPTION AND ANALYSIS OF THE REGULATORY PROCESS

Throughout this book, we have used in parallel the classical description based on ordinary differential equations and the discrete method called "kinetic logic". The idea behind the discrete approach is that in many cases any attempt to treat a biological system in really quantitative terms is illusory, and it is often more useful to have a general view of the essential dynamic possibilities of a system rather than to know the quantitative details of its behavior in particular conditions. We agree with Thom² that one can be at the same time qualitative and rigorous. What we are really interested in is the qualitative essence of a system.

In kinetic logic, instead of relating the state of a system "at time $t + 1$ " to its state "at time t " (the synchronous description), we relate logical functions describing the *evolution* of the system to variables describing its *state*. Thus, for each state of the system described by a variable vector $abc...$, we associate a function vector $ABC...$ (the *image* of the variable vector) that describes its evolution. More specifically, the *differences* between vector abc and vector ABC indicate to which orders the system is subject when it is in state abc . For example, in the loop:



for $abc = 001$, we have $ABC = 010$; there is an order to switch c from 1 to 0 and an order to switch b from 0 to 1. Thus, the image ABC of state abc would be the state following if all orders to which the system is subject when it is in state abc were executed simultaneously. However, this is not normally the case. Among the orders to which the system is subjected in state $abc...$, one is executed first, thus leading the system to a new state in which part of the orders present in state abc may be canceled, and new orders may arise. In the present case, from 001, instead of going to its image 010 by synchronously switching c off and b on, the system will usually go either to 000 or 011. Note that in the first case the order to switch on b persists, and there is a new order (to switch on a); in the second case, the order to switch off c is canceled.

Formally, the evolution of the system is described as a logical iteration, but this iteration is neither a classical "parallel" iteration (*à la* Jacobi), in which all variables with an order to change do so simultaneously, nor a classical "series" iteration (*à la* Gauss-Seidel), in which variables change their value one at a time in a predetermined order. In our description, only

one order is executed at a time; which one depends on the relative values of time delays or linear combinations of them.

Thus, a distinctive feature of our discrete description is its fully *asynchronous* character, without which systems would be condemned to follow a single linear pathway, without any possibility of choice. More specifically, in our description, a given logical structure (graph of interactions) can often generate a number of distinct pathways; which one is followed depends on the time delays. Of course, if we assign a fixed value to each time delay, the system will follow a determined pathway. However, in a cell population, it is reasonable to assign to each delay an average value and a distribution rather than a well-defined value, permitting cells as similar as possible to follow different pathways. This is how *stochastic* elements can enter our description.

Note that, in our description, systems are treated as special cases of *asynchronous automata*. In fact, our formalism permits the description of asynchronous automata in general, and not only in cases representing biological applications.

With the help of Van Ham and of Richelle, we introduced variable with more than two values whenever necessary. The simple (and *a posteriori* obvious) criterion for the number of values a variable should have was the number of targets of the corresponding element. If it acts at n points, up to n distinct threshold values might be required, and thus up to $n + 1$ logical levels.

More recently, Snoussi introduced "logical parameters", which take into account the relative weight of each term of a logical expression. In addition, Snoussi's description solves the problems encountered in assigning values to multivalued logical functions. At this stage, our discrete formalization permits a flexible qualitative description of complex systems. It is rewarding to observe that this description is qualitatively extremely similar to that given by systems of nonlinear differential equations using sigmoid interactions. Some of the stable steady states of the differential description are seen in the logical description as logical "stable states". As briefly mentioned in the last section of Chapter 8, we recently discovered that the other steady states of the differential description can also be identified on logical grounds if one includes the thresholds as additional logical values in the description.

From a more general viewpoint, our logical description is effective for any system that can be described by an oriented, signed graph. In fact, the method was developed for the study of gene networks, but it soon turned out to be usable not only in other fields of biology (e.g., immunology, neurobiology, and virology³), but also outside biology. Nicolis⁴ and subsequently, Dee and Ghil⁵ have applied kinetic logic to climatology,* de Palma and Boon⁶ to urbanism, and de Palma, Stengers, and Pahaut⁷ to decision making.

IV. SOME PERSPECTIVES

In some fields of biology, we are beginning to understand molecular mechanisms in great detail. Unfortunately, in many cases we feel that we know the cogwheels, but not the clock. This is why it is so necessary to be in a position to study complex networks with intertwined feedback loops. Kinetic logic, which provides such a tool, has progressed enormously *during* (and, as a matter of fact, partly because of) the writing of this book.

We are now able, from a graph of interactions, to provide a limited number of qualitatively different submodels, each characterized by a specific set of values for the logical

* The variant proposed by Ghil uses as many delays as there are interactions. However, it uses the same value for the "on" and "off" delays. Another difference is that once an order has been given, it will be executed after the characteristic time delay, even if there is a counter order. This results in complex behavior, very different from that given by the differential description.

parameters. For each submodel, we know the essential aspects of the dynamics, and we can write a system of nonlinear differential equations that, using real parameters inspired from the logical ones, will behave essentially the same as its discrete homologue. Moreover, we can now, on logical grounds alone, identify the steady states of our systems and predict their location and essential properties in the differential system.

In fact, Snoussi's generalization became operative only two years ago (in 1987) and the logical identification of all steady states is even more recent. It is thus not surprising that the new methods have been applied so far in only a few concrete cases — as the reader will have realized. It will be especially interesting to apply these new methods to systems involving "strange" (chaotic) attractors.

The most unusual, and perhaps the most fecond, feature of the logical description is that instead of trying to give a *linear* approximation of nonlinear systems, we take the diametrically opposite attitude and use an *infinitely nonlinear* caricature. Whereas linear approximations are appropriate only in the close vicinity of steady states, the infinitely nonlinear caricature provides a qualitatively correct account of the behavior of systems comprising sufficiently nonlinear feedback loops.

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Appendix 1

HOW TO FIND A STEADY STATE VALUE BY ITERATION

When an equation is written:

$$H(x) = 0 \quad (1)$$

its solution can be visualized as the intersects of the curve $y = H(x)$ with the "curve" $y = 0$, i.e., the x axis (Figure 1a). Equation 1 can also be written in the form:

$$x = F(x) \quad (2)$$

where $F(x) = H(x) + x$. The solutions are the values of x such that x itself and $F(x)$ are equal. They can thus be visualized as the intersects of $y = F(x)$ and of $y = x$, i.e., the bissectrix of the positive quadrant (see Figure 1b). It is obvious that Equations 1 and 2 have the same solutions. The form of Equation 2, however, is often more convenient for numerical approximations.

Let us start from an arbitrary value x_1 , calculate $F(x_1)$ and use this as a new estimate of x , $x_2 = F(x_1)$, then calculate $F(x_2)$ and use it as a third estimate of x . More generally:

$$x_{n+1} = F(x_n) \quad (3)$$

Such a relation, called a *recursion formula*, generates an *iterative process* (discovered long ago by Euler). This iteration is illustrated in Figure 2 by the successive arrows starting from x_1 . From x_1 , a vertical line is drawn to the curve $y = F(x)$; the intersect has coordinates $(x_1, F(x_1))$ or, equivalently, (x_1, x_2) . If one now draws a horizontal line to the bissectrix, the intersect has the coordinates (x_2, x_2) . A vertical line drawn through this point will cross $y = F(x)$ at the point $[x_2, F(x_2)]$ or, equivalently, (x_2, x_3) , and so on. It can be seen that in this case the iteration rapidly approaches one of the intersects between $y = F(x)$ and $y = x$; that is, one of the solutions of the equation $x = F(x)$.

The process shown in Figure 2 is readily done with a pocket calculator, in which one "feeds" function $F(x)$ the arbitrary initial value x_1 , then feeds it the result $F(x_1)$ used as x_2 , and so on. In the case considered, and using $x_1 = 1.1$, the successive values are

$x_1 = 1.1$	$x_9 = 1.927\ 514\ 385$
$x_2 = 1.233\ 866\ 179$	$x_{10} = 1.927\ 553\ 356$
$x_3 = 1.481\ 842\ 538$	$x_{11} = 1.927\ 560\ 415$
$x_4 = 1.754\ 454\ 578$	$x_{12} = 1.927\ 561\ 693$
$x_5 = 1.886\ 512\ 250$	$x_{13} = 1.927\ 561\ 924$
$x_6 = 1.919\ 661\ 005$	$x_{14} = 1.927\ 561\ 966$
$x_7 = 1.926\ 114\ 472$	$x_{15} = 1.927\ 561\ 974$
$x_8 = 1.927\ 299\ 284$	$x_{16} = 1.927\ 561\ 975$

The value of x to nine decimals does not change any more after 15 iterations. One can easily check that any initial value above 1 would lead to the same point.

The intersects of the two curves, which are, as already mentioned, the solutions of the equation $x = F(x)$, are also called *fixed points* of the recursion $x_{n+1} = F(x_n)$ because if one starts from such a point x^0 , there is no further change; $x^0 = F(x^0)$. In our example, there are three fixed points, the one just calculated ($x = 1.927\dots$), $x = 0$, and $x = 1$.

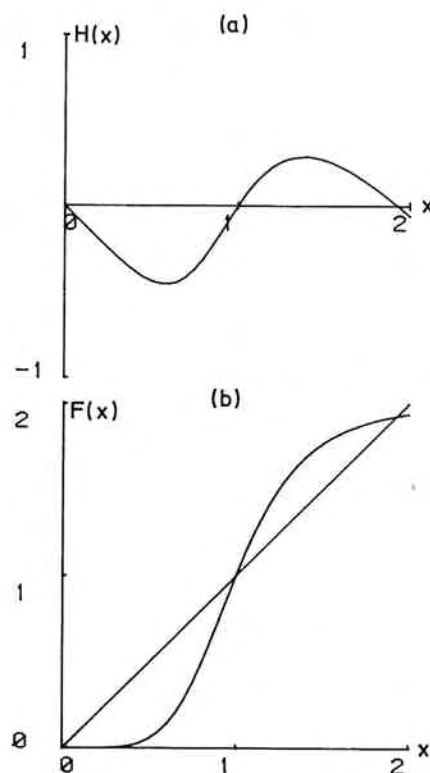


FIGURE 1. A plot of $H(x) = \frac{2x^5}{1+x^5} - x$ as a function of x . The solutions of equation $H(x) = 0$ are given by the intersects of the curve with the x axis. (b) A plot of $F(x) = \frac{2x^5}{1+x^5}$ as a function of x . The solutions of equation $x = F(x)$ (equivalent to $H(x) = 0$) are the values of x at the intersects of $F(x)$ with the bissectrix.

If, instead of starting from 1.1, we had started from 0.9, the iteration would have led us to another fixed point. The successive values given by the pocket calculator are

$$\begin{aligned} x_1 &= 0.9 \\ x_2 &= 0.742\,525\,888 \\ x_3 &= 0.368\,297\,644 \\ x_4 &= 0.013\,461\,446 \\ x_5 &= 0.000\,000\,001 \\ x_6 &= 1 \times 10^{-45} \\ x_7 &= 0.0 \end{aligned}$$

In fact, one can show that any positive x_1 less than 1 would lead to the same fixed point $x = 0$. Thus the recursion chosen can be used to calculate two of the steady-state values, but not the third. We will soon see how to calculate this third steady state as well.

We will first consider two other examples (Figure 3a and b), both decreasing functions. In the first (Figure 3a), the iteration converges toward the unique fixed point. Note that the convergence is spiral here, whereas it was monotonic in Figure 2. This is because the curve is increasing in Figure 2, decreasing in Figure 3. Expressing $F(x)$ on a pocket calculator and

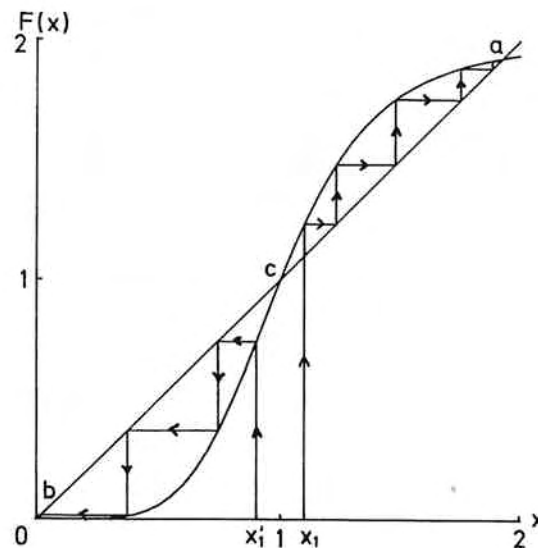


FIGURE 2. Iteration

$$x_{n+1} = \frac{2x_n^5}{1 + x_n^5}$$

using $x_1 = 1.1$ and $x'_1 = 0.9$. It can be seen that the fixed points a and b, but not c, can be reached by this iteration process.

choosing $x_1 = 0.1$ (far from the steady state), $x_2 = F(x_1)$, etc., the successive values converge toward 1, reached to nine decimals after 32 iterations.

In contrast, the iteration in Figure 3b does not converge; it approaches a two-element cycle. The pocket calculator, starting from 1.1 (which is close to the steady state), gives:

$$\begin{aligned} x_1 &= 1.1 \\ x_2 &= 0.766\ 133\ 821 \\ x_3 &= 1.582\ 339\ 928 \\ x_4 &= 0.183\ 155\ 063 \\ x_5 &= 1.999\ 587\ 869 \\ x_6 &= 0.060\ 666\ 648 \\ x_7 &= 1.999\ 998\ 356 \\ x_8 &= 0.060\ 606\ 302 \\ x_9 &= 1.999\ 998\ 364 \\ x_{10} &= 0.060\ 606\ 301 \\ x_{11} &= 1.999\ 998\ 364 \end{aligned}$$

and so on.

Thus, of the five fixed points examined in our examples, three (a and b of Figure 2, d of Figure 3a) are attractive, and two (c of Figure 2 and e of Figure 3b) are repulsive. On what does this depend? Simply on the *slope* of the curve $F(x)$ at the fixed point considered. If the absolute value of the slope is less than 1, the process will converge, but our iteration will not converge if the slope is greater than 1 (as for point c) or less than -1 (as for point e).

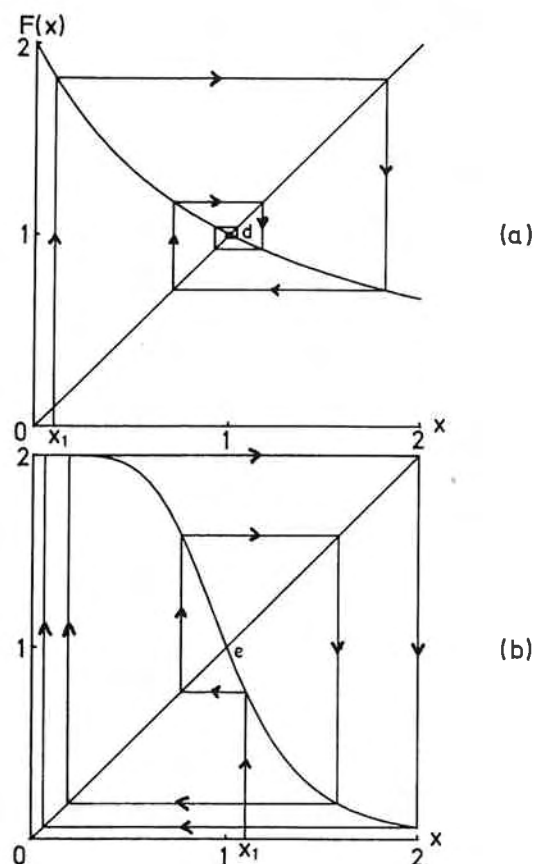


FIGURE 3.

a) Iteration $x_{n+1} = \frac{2}{1+x_n}$

Iteration converges spirally toward the unique fixed point.

b) Iteration $x_{n+1} = \frac{2}{1+x_n^5}$

The iteration fails to converge and tends toward a cycle.

How can one reach by iteration a point that is repulsive? A very simple method¹ is as follows. Instead of $x = F(x)$, one can write $x - Ax = F(x) - Ax$ or

$$x = \frac{F(x) - Ax}{1 - A} = G(x) \quad (A \neq 1) \quad (4)$$

The solutions of Equation 4 are obviously the same as those of Equation 2, which is a special case of Equation 4 with $A = 0$. However, the *shape* of $G(x)$ and, in particular, its slope at its intersects with the bisectrix depend on the value of A . For appropriate values of A , the iteration

$$x_{n+1} = \frac{F(x_n) - Ax_n}{1 - A} = G(x_n) \quad (5)$$

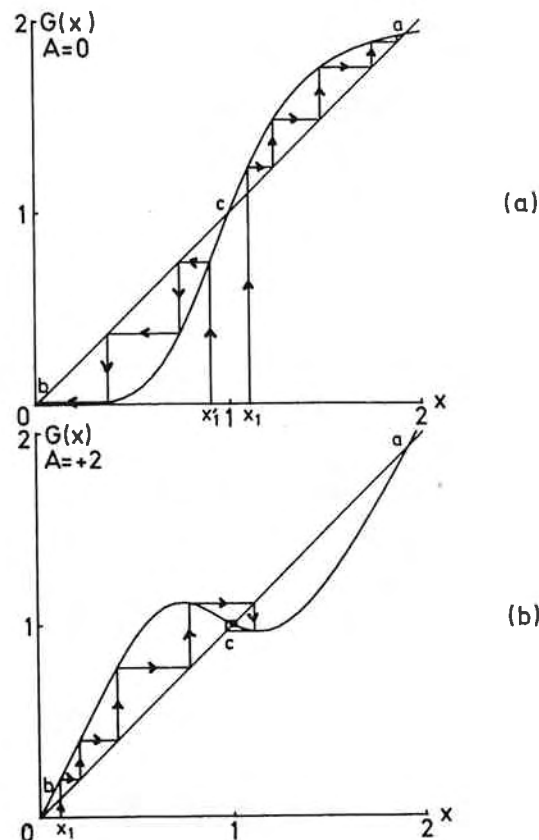


FIGURE 4. To reach the intermediate fixed point of Figure 2, we use the recursive formula

$$x_{n+1} = \frac{F(x_n) - Ax_n}{1 - A}$$

With $A = 0$ (Figure 4a), one again finds the situation of Figure 2. With $A = +2$ (Figure 4b), the process converges toward the fixed point $x^0 = 1$ (but the other fixed points become repulsive).

will reach a fixed point that was repulsive for $A = 0$. For instance, for point c (Figure 4a), using $A = +2$ and starting from $x = 0.1$, the iteration converges toward 1. For point e (Figure 5b) with $A = -2$ and starting from $x = 0.1$, the iteration converges toward the fixed point 1.

How did we choose the values $A = +2$ for point c, and $A = -2$ for point e? It can be shown that the *optimal* value of A (that giving the most rapid convergence) is the slope of $F(x)$ at the fixed point — in other words, the derivative $F'(x^0)$. Of course, we cannot know $F'(x^0)$ unless we already know x^0 , but what we know is that in the first case the classical iteration failed because the slope was greater than 1 and in the second case it failed because the slope was less than -1 . Thus, in the first case (c), one has to use a “sufficient” positive value for A and in the second case (e), a “sufficient” negative value. It can be shown *a posteriori* that for a point c the optimal value of A is $+2.5$, but that any value greater than 1.75 would ensure convergence, and for point e the optimal value is -2.5 , but any value less than -0.75 would ensure convergence.

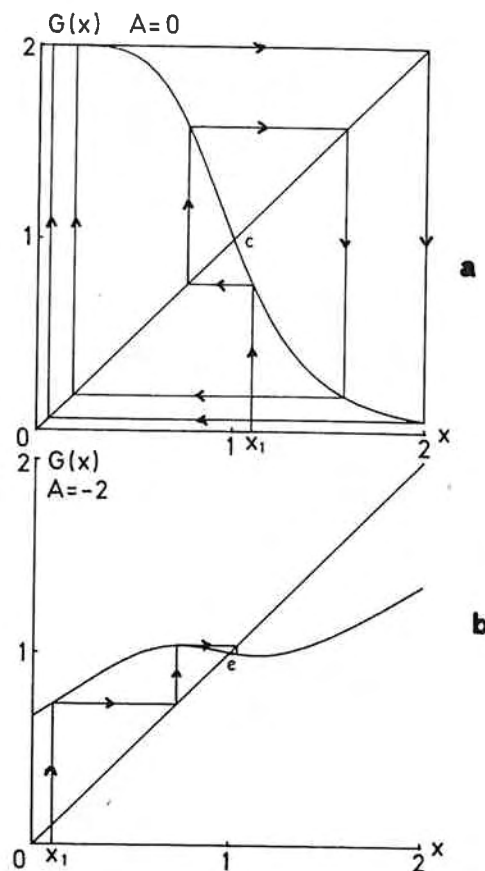


FIGURE 5. To reach the fixed point (e) of Figure 3b, we again use the formula:

$$x_{n+1} = \frac{F(x_n) - Ax_n}{1 - A}$$

this time with A negative ($A = -2$). See Figure 5b.

In practice, when a fixed point is repulsive for $A = 0$ using the classical recursion relation 3, we use recursion 5 with a positive or negative value of A , depending on whether the slope of $F(x)$ at this point is itself positive or negative. If the iteration still does not converge, one can increase the absolute value of A . One can also use recursion 5 to increase the rate of convergence of a slowly convergent process. For example, for the equation of Figure 3a, the fixed point $x^0 = 1$ (to nine decimals) is only reached after 32 iterations with the classical procedure ($A = 0$) starting from $x_1 = 0.1$, but already after four iterations if $A = -0.5$.

The method just described for one-variable systems can be extended to n -variable systems, but such considerations are beyond the scope of this book. Some indications can be found in Thomas et al.¹ Suffice it to say that for n variables, A is replaced by an $n \times n$ matrix that is a caricature of the Jacobian matrix at the steady state one is trying to reach. The method is currently used to determine the steady-state values of n -variable nonlinear differential systems by solving their steady-state equation systems.

Since the solutions of the steady-state equation system are also fixed points of the corresponding recursive equations, one might at first think that the physical stability of a steady

state can be inferred from the attractive vs. repulsive character of the corresponding fixed point. The reality is not so simple. For instance, the unique steady state ($x = 1$) of

$$\frac{dx}{dt} = \frac{2}{1+x^5} - x$$

is stable (see the linear stability analysis, Appendix 3). However, if from the steady-state equation $x = \frac{2}{1+x^5}$, one derives the recursive formula $x_{n+1} = \frac{2}{1+x_n^5}$, its unique fixed point $x = 1$ is found to be repulsive (see Figure 3b).

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Appendix 2

TRAJECTORIES AND EVOLUTION

As we have repeatedly pointed out, systems of nonlinear differential equations usually cannot be integrated analytically. However, for any given set of parameter values, trajectories and evolution can be computed numerically. The basic formula is Euler's iteration:

$$x_{n+1} = x_n + hH(x_n)$$

in which, as above, we write $H(x)$ for $\frac{dx}{dt}$. This formula is a linear approximation. Admittedly, it can be made to come arbitrarily close to the real situation by using an arbitrarily small step h . However, in practice, the step cannot be made too small without running into far too much computer time for the enormous number of steps required.

This is why various improvements of the Euler formula are used. The simplest is the so-called "improved Euler" method. A more elaborate version is the Runge-Kutta method. Both are described by Kreyszig.¹

In this appendix, we have chosen a simple system that can be integrated analytically and have used the simple and improved Euler methods. In Figure 1 is shown a comparison of the

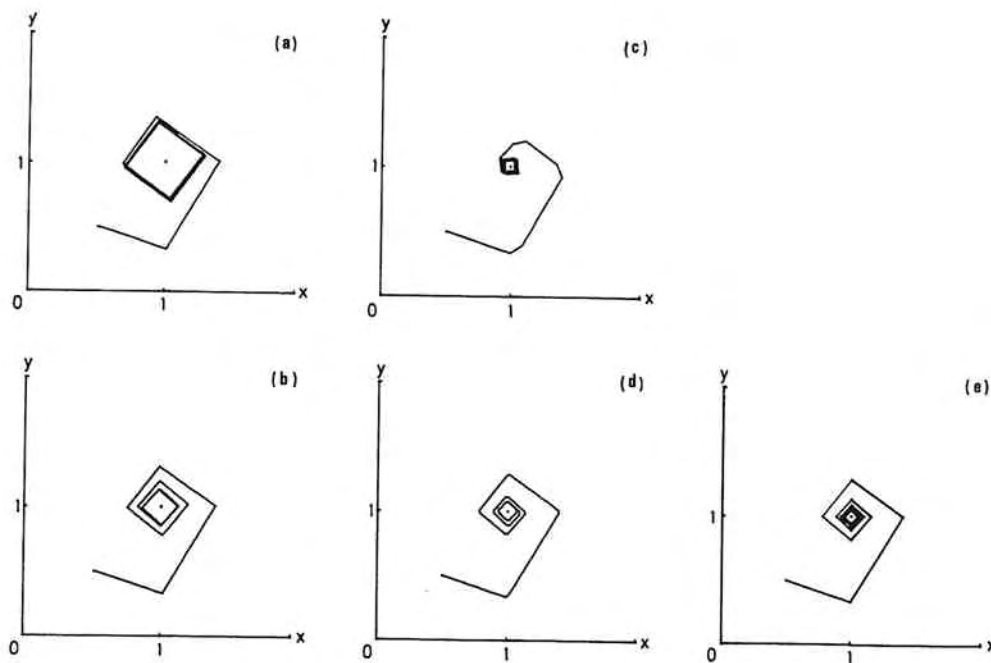


FIGURE 1. A comparison of Euler, improved Euler, and analytic trajectories for the system

$$\frac{dx}{dt} = \frac{2}{1+y^n} - x$$

$$\frac{dy}{dt} = \frac{2x^n}{1+x^n} - y$$

(with $n = \infty$). (a) Euler iteration, step 0.1; (b) Euler iteration, step 0.02; (c) improved Euler iteration, step 0.1; (d) improved Euler iteration, step 0.02; (e) analytic.

Euler method with two different steps, 0.1 and 0.02 (a and b), the improved Euler method with the same two steps (c and d), and the analytic result (e).

Whatever method is used, the successive positions of the system are computed for 1, 2, 3, ..., n time intervals of length h . Trajectories are obtained by plotting the successive values of the vector x . For a two-variable system, these values are plotted in the plane of the variables, x_1x_2 (or xy). For systems with three or more variables, one usually plots projections on the plane of two chosen variables. The evolution in time is shown by the successive values of each variable plotted as a function of time.

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Appendix 3

A LITTLE MORE ABOUT LINEAR STABILITY ANALYSIS

The idea in stability analysis is to perturb the system by removing it slightly from its steady state, then to determine whether the perturbation grows or regresses. In the close vicinity of the steady state, it is convenient, instead of dealing with the concentrations themselves, to consider the size of the perturbation, i.e., the difference between the concentration of a product x and its steady state value x^0 . Let us first consider a one-variable system. Close to steady state, the difference $\xi = x - x^0$ is small enough that one can limit the expression of $H(x)$, or $H(x^0 + \xi)$, to the linear term of its Taylor expansion around x^0 .

The classical development can be recalled as follows. Assume that $H(x)$, or $H(x^0 + \xi)$, can be written as a polynomial in ξ : $H(x^0 + \xi) = a_0 + a_1\xi + a_2\xi^2 + a_3\xi^3 + \dots$. The values of a_0 , a_1 , a_2 , ... are easily derived by noticing that:

$$H(x^0) = a_0$$

$$\frac{dH(x)}{dx} = \frac{dH(x^0 + \xi)}{d\xi} = a_1 + 2a_2\xi + 3a_3\xi^2 + \dots$$

and thus $\frac{dH(x^0)}{d\xi} = a_1$

$$\frac{d^2H(x)}{d\xi^2} = \frac{d^2H(x^0 + \xi)}{d\xi^2} = 2a_2 + 6a_3\xi + \dots$$

and thus $\frac{d^2H(x^0)}{d\xi^2} = 2a_2$, or $a_2 = \frac{1}{2} \frac{d^2H(x^0)}{d\xi^2}$, etc.

This generates the Taylor series:

$$H(x^0 + \xi) = H(x^0) + \frac{dH(x^0)}{d\xi} \xi + \frac{1}{2} \frac{d^2H(x^0)}{d\xi^2} \xi^2 + \dots + \frac{1}{n!} \frac{d^n H(x^0)}{d\xi^n} \xi^n + \dots$$

Close to the steady state, the perturbation ξ is small enough that the terms of higher order can be neglected. In addition, because x^0 is a steady state, we have $H(x^0) = 0$. Therefore:

$$H(x) = H(x^0 + \xi) \approx \frac{dH(x^0)}{d\xi} \xi$$

But

$$H(x) = \frac{dx}{dt} = \frac{d(x^0 + \xi)}{dt} = \frac{d\xi}{dt}$$

and consequently:

$$\frac{d\xi}{dt} \approx \frac{dH(x^0)}{d\xi} \xi \quad (2)$$

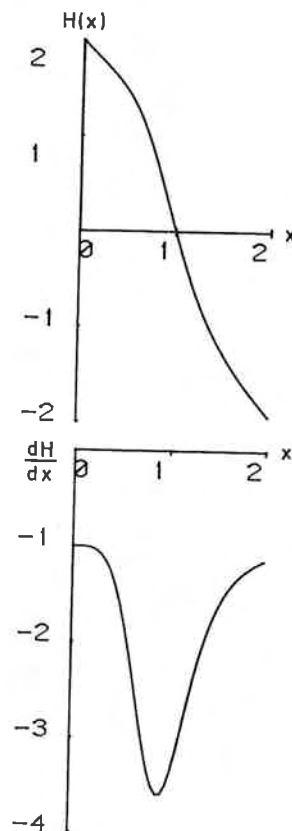


FIGURE 1. Plot of $\frac{dx}{dt}$, or $H(x)$, and of $\frac{dH}{dx}$ as functions of x for the one-variable system $H(x) = kF(x) - kx$. Specifically, we take:

$$H(x) = \frac{2}{1+x^5} - x$$

$$\frac{dH}{dx} = \frac{-10x^4}{(1+x^5)^2} - 1$$

As the unique steady-state value is $x^0 = 1$ (see Appendix 1), $\left(\frac{dH(x^0)}{dx}\right)_{x^0} = -3.5$, the slope of $H(x)$ at x^0 . In the present case, the unique steady state is stable because (dH/dx) is negative; any small perturbation will thus regress with time.

Thus, near a steady state, the nonlinear differential equation $\frac{dx}{dt} = H(x)$ can be linearized to $\frac{d\xi}{dt} = \omega\xi$ (with $\omega = \frac{dH(x^0)}{dx}$), a linear differential equation that has solutions of the simple form $\xi = \xi_0 e^{\omega t}$. This implies that if the system drifts away from the steady state by a small amount, it will return toward it or depart further from it, according to whether $\omega = \frac{dH(x^0)}{dx}$ is negative or positive. In other words, in a one-variable system, a steady state x^0 will be *stable* or *unstable*, depending simply on the sign of $\frac{dH(x^0)}{dx}$, the slope of function $H(x)$ at the steady state (see Figure 1).

We now consider a two-variable system,

$$\frac{dx}{dt} = H_x(x, y)$$

$$\frac{dy}{dt} = H_y(x, y)$$

As just shown for a one-variable system, one can reason in terms of the small perturbations $\xi = x - x_0$ and $v = y - y_0$. In the immediate vicinity of a steady state (x^0, y^0) , $\frac{d\xi}{dt}$ and $\frac{dv}{dt}$, which equal $\frac{dx}{dt}$ and $\frac{dy}{dt}$, respectively, can be approximated by linear functions of ξ and v themselves:

$$\begin{aligned} \frac{d\xi}{dt} &= a_{11}\xi + a_{12}v \\ \frac{dv}{dt} &= a_{21}\xi + a_{22}v \end{aligned} \quad (3)$$

in which the coefficients are partial derivatives evaluated at the steady state:

$$\begin{aligned} a_{11} &= \left(\frac{\partial H_x}{\partial x} \right)_{x^0, y^0} & a_{12} &= \left(\frac{\partial H_x}{\partial y} \right)_{x^0, y^0} \\ a_{21} &= \left(\frac{\partial H_y}{\partial x} \right)_{x^0, y^0} & a_{22} &= \left(\frac{\partial H_y}{\partial y} \right)_{x^0, y^0} \end{aligned}$$

In the one-variable system, the unique coefficient of Equation 2 was the derivative $\frac{dH(x^0)}{dx}$. In a two- (or more) variable system, the role of the derivative is played by the Jacobian matrix:

$$\begin{bmatrix} \frac{\partial H_x}{\partial x} & \frac{\partial H_x}{\partial y} \\ \frac{\partial H_y}{\partial x} & \frac{\partial H_y}{\partial y} \end{bmatrix}$$

It can be shown (see, for instance, Reference 1) that systems of homogeneous linear differential equations like Equation(s) 3 have particular solutions of the form:

$$\xi = \xi_0 e^{\omega t}; v = v_0 e^{\omega t}$$

hence,

$$\frac{d\xi}{dt} = \omega \xi_0 e^{\omega t}, \quad \frac{dv}{dt} = \omega v_0 e^{\omega t},$$

in which ξ_0 and v_0 are related constants and ω another constant to be determined. Substituting these values in Equation 3, canceling out the factor $e^{\omega t}$, and rearranging, we obtain:

$$\begin{aligned}(a_{11} - \omega)\xi_0 + a_{12}v_0 &= 0 \\ a_{21}\xi_0 + (a_{22} - \omega)v_0 &= 0\end{aligned}\tag{4}$$

Such a system of equations can be satisfied by nonzero values of ξ_0 and v_0 only if the determinant of the coefficients is 0*:

$$\begin{vmatrix} a_{11} - \omega & a_{12} \\ a_{21} & a_{22} - \omega \end{vmatrix} = 0$$

We therefore choose those values of ω that satisfy this condition. Development of the determinant yields the relation:

$$\omega^2 - (a_{11} + a_{22})\omega + a_{11}a_{22} - a_{12}a_{21} = 0\tag{5}$$

which is called the "characteristic equation" of the system. In the present case (two-variable system), it is a second-degree equation in ω . Thus, ω usually has two distinct values, ω_1 and ω_2 . Substituting first ω_1 and then ω_2 in Equations 4, one finds the corresponding permissible pairs ξ_{01}, v_{01} , and ξ_{02}, v_{02} .

The general solution of the system of linear differential Equations 3 is a linear combination of the particular solutions with ω_1 and ω_2 :

$$\begin{aligned}\xi(t) &= C_1\xi_{01}e^{\omega_1 t} + C_2\xi_{02}e^{\omega_2 t} \\ v(t) &= C_1v_{01}e^{\omega_1 t} + C_2v_{02}e^{\omega_2 t}\end{aligned}\tag{6}$$

where C_1 and C_2 are arbitrary constants that depend on the initial perturbation.

From Equation 6, it is apparent that a perturbation will regress in time — and consequently the steady state will be stable — only if *both* ω_1 and ω_2 (or their real parts) are negative. In this case, all terms will vanish with time.

When the roots are complex conjugates, we can write $\omega = \alpha \pm i\beta$, with α and β real. From the Euler formula for complex exponents, we have

$$e^{\omega t} = e^{(\alpha \pm i\beta)t} = e^{\alpha t}(\cos \beta t \pm i \sin \beta t)$$

It should be stressed that even when ω is complex, $\xi(t)$ and $v(t)$, which represent the actual perturbation at time t , are real. Since $\xi_{01}, v_{01}, \xi_{02}, v_{02}$ become complex whenever ω is complex, one must allow C_1 and C_2 to be complex. Equation 6 then takes the form:

$$\xi(t) = A_1 e^{\alpha t} (\cos \beta t + \Psi_1)$$

$$v(t) = A_2 e^{\alpha t} (\cos \beta t + \Psi_2)$$

in which the A s and Ψ s depend both on the coefficients a_{ij} and on the initial perturbation. From these equations, it can be seen that when ω is complex, the perturbation will oscillate.

* Under these conditions, the two equations are not independent; one is a multiple of the other.

If α , the real part of the roots, is negative, the perturbation will follow a damped periodic regression back toward the steady state, which is a stable focus, and if α is positive, the perturbation will follow an increasing periodic departure from an unstable focus. For the marginal case when $\alpha = 0$ and the roots are pure imaginary numbers, the system would, in principal, oscillate indefinitely at a distance from the steady state (here called *center*) which depends on the initial state.

We recapitulate the above remarks as follows. For a one-variable system, the equivalent of the characteristic equation has a simple root, and a steady state is stable or unstable depending simply on whether the root is negative or positive, respectively. For two-variable systems, on the other hand, the characteristic equation has two roots that, if real, can be both positive, both negative, or one positive and one negative; if complex conjugates, their real part can be positive or negative. A steady state is stable only if both roots (or their real parts) are negative. Furthermore, the approach to or departure from the steady state is direct or periodic depending on whether the roots are real or complex. If the roots have opposite signs, the steady state (which is unstable) is called a *saddle point* (see Chapter 6, Example 2).

A second-degree equation can always be written in the form:

$$\omega^2 - S\omega + P = 0 \quad (7)$$

in which S is the *sum* and P the *product* of the roots.* Comparing Equation 7 with Equation 5, it can be seen that:

$$S = a_{11} + a_{22}$$

and

$$P = a_{11}a_{22} - a_{12}a_{21}$$

Furthermore, the roots are real iff $S^2 - 4P \geq 0$.

The various cases that can occur for a two-variable system are readily visualized by drawing the curve $S^2 - 4P = 0$ in the S - P plane (Figure 2). P positive (north part of the plane) implies that the roots have the same sign (or are complex conjugates). In the NE quadrant (S positive), the roots are both positive (or the real part is positive), whereas in the NW quadrant (S negative), they are both negative (or the real part is negative). $S^2 - 4P$ is negative (and thus the roots are complex) above the parabola, and positive (real roots) below the parabola. A steady state that falls in the north half can be *stable* (in the NW quadrant) or *unstable* (in the NE quadrant); it can be a *focus* (above the parabola) or a *node* (below the parabola).

P negative implies that the roots have opposite signs. In this case, the steady state is attractive along one direction (corresponding to the negative root) and repulsive elsewhere. This type of steady state, called a *saddle point*, will be described in more detail apropos of positive feedback loops (see also Example 3 below).

More generally, for an n -variable system, the characteristic equation is of degree n , and ω thus usually has n distinct values. A steady state is stable only if all values of ω (or their real parts) are negative. In addition, complex roots will give rise to periodic approaches to or departures from the steady state. As we shall see, in systems with more than two variables, there is an increasing variety of steady states.

* This can be seen by considering a second-degree equation with roots ω_1 and ω_2 . The equation can be written: $(\omega - \omega_1)(\omega - \omega_2) = 0$ or $\omega^2 - (\omega_1 + \omega_2)\omega + \omega_1\omega_2 = 0$.

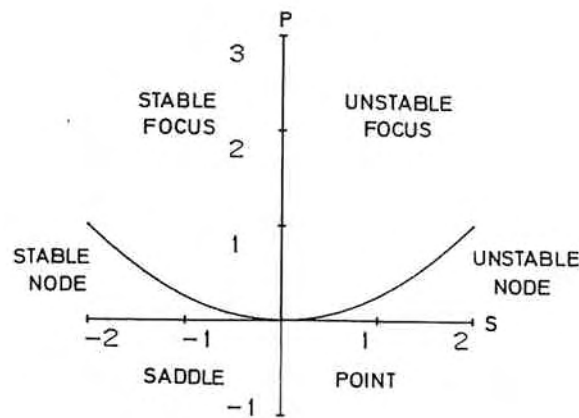


FIGURE 2. The nature of steady states in two-variable systems. The location of a steady state in S-P space with respect to the axes and the parabola $S^2 - 4P = 0$ indicates the nature of the steady state. S is the sum and P is the product of the roots of the characteristic equation.

Example 1. Let us treat the two-element negative loop $x \begin{array}{c} \xrightarrow{+} \\ \xleftarrow{-} \end{array} y$ described in Chapter 6, Sections V—VIII.

$$H_x = \frac{dx}{dt} = \frac{2}{1 + y^5} - x$$

$$H_y = \frac{dy}{dt} = \frac{2x^5}{1 + x^5} - y$$

The elements of the Jacobian matrix are

$$\frac{\partial H_x}{\partial x} = -1$$

$$\frac{\partial H_x}{\partial y} = \frac{-10y^4}{(1 + y^5)^2}$$

$$\frac{\partial H_y}{\partial x} = \frac{+10x^4}{(1 + x^5)^2}$$

$$\frac{\partial H_y}{\partial y} = -1$$

In this system, the unique steady state (x^0, y^0) has the coordinates (1, 1) (see Appendix 1). Thus:

$$a_{11} = \left(\frac{\partial H_x}{\partial x} \right)_{x^0, y^0} = -1$$

$$a_{12} = \left(\frac{\partial H_x}{\partial y} \right)_{x^0, y^0} = -2.5$$

$$a_{21} = \left(\frac{\partial H_y}{\partial x} \right)_{x^0, y^0} = +2.5$$

$$a_{22} = \left(\frac{\partial H_y}{\partial y} \right)_{x^0, y^0} = -1$$

The linearized system is then:

$$\frac{d\xi}{dt} = -\xi - 2.5\upsilon$$

$$\frac{dv}{dt} = +2.5\xi - v$$

and the characteristic equation is

$$\begin{vmatrix} -1 - \omega & -2.5 \\ +2.5 & -1 - \omega \end{vmatrix} = 0$$

or

$$\omega^2 + 2\omega + 7.25 = 0$$

The roots are $\omega = -1 \pm 2.5i$. The real part is negative, and the steady state is therefore stable. Since there is a complex part, the steady state is approached periodically and is a stable focus (see Chapter 6, Figure 5a).

Example 2. We take the same system as in Example 1, except for different parameter values:

$$H_x = \frac{dx}{dt} = \frac{2}{1 + y^5} - 3x$$

$$H_y = \frac{dy}{dt} = \frac{2x^5}{1 + x^5} - y$$

The elements of the Jacobian matrix are the same as in Example 1, except that:

$$\frac{\partial H_x}{\partial x} = -3$$

The steady-state values (see Appendix 1) are

$$x^0 = 0.666$$

$$y^0 = 0.232$$

and

$$a_{11} = \left(\frac{\partial H_x}{\partial x} \right)_{x^0, y^0} = -3$$

$$a_{12} = \left(\frac{\partial H_x}{\partial y} \right)_{x^0, y^0} = -0.029$$

$$a_{21} = \left(\frac{\partial H_y}{\partial x} \right)_{x^0, y^0} = 1.539$$

$$a_{22} = \left(\frac{\partial H_y}{\partial y} \right)_{x^0, y^0} = -1$$

The characteristic equation is thus $\omega^2 + 4\omega + 3.045 = 0$. There are two real roots, both negative: -1.022 and -2.977 . The steady state is thus a stable node, approached directly (see Chapter 6, Figure 5b).

Example 3. This is the two-element positive loop we considered in Chapter 12,



$$H_x = \frac{dx}{dt} = \frac{2}{1 + y^5} - x$$

$$H_y = \frac{dy}{dt} = \frac{2}{1 + x^5} - y$$

The elements of the Jacobian matrix are

$$\frac{\partial H_x}{\partial x} = -1$$

$$\frac{\partial H_x}{\partial y} = \frac{-10y^4}{(1 + y^5)^2}$$

$$\frac{\partial H_y}{\partial x} = \frac{-10x^4}{(1 + x^5)^2}$$

$$\frac{\partial H_y}{\partial y} = -1$$

In this case, there are three steady states (see Appendix 1):

$$(x^0, y^0) = (0.061, 2.00)$$

$$(x^{0'}, y^{0'}) = (1, 1)$$

$$(x^{0''}, y^{0''}) = (2.00, 0.061)$$

The first and third steady states are readily shown to be stable nodes. Let us look at the second steady state. The values of the coefficients at (1, 1) are

$$a_{11} = -1$$

$$a_{12} = -2.5$$

$$a_{21} = -2.5$$

$$a_{22} = -1$$

and the characteristic equation is

$$\omega^2 + 2\omega - 5.25 = 0$$

The roots are +1.5 and -3.5. This steady state is therefore a saddle point and thus, unstable.

REFERENCE

1. Kreyszig, E., *Advanced Engineering Mathematics*, 5th ed., John Wiley & Sons, New York, 1983.

ASYNCHRONOUS VS. SYNCHRONOUS DESCRIPTION

INTRODUCTION

Kauffman¹ studied the behavior of large, randomly constructed networks of "binary genes". Assuming that the networks are *synchronous* (see below), he showed that if each "gene" is directly affected by two or three other "genes", then such random networks behave with great order and stability; in particular, they follow surprisingly short cycles.

The synchronous treatment can be described as follows:¹ "If the system is placed in some state at time T , then at time $T + 1$ each gene scans the present values of each of its K inputs, consults its Boolean function, and assumes the value specified for that input configuration." As pointed out by Kauffman, this attitude implies that "the net passes from a state to *one* subsequent state; therefore, although two states may converge onto a single subsequent state, no state may diverge onto two subsequent states". This is an oversimplification in general, and in particular for biological systems that have to differentiate. On the other hand, it has the virtue of simplicity, and it was justified to adopt this attitude for a first analysis. Very interesting work has been done using this synchronous description.²

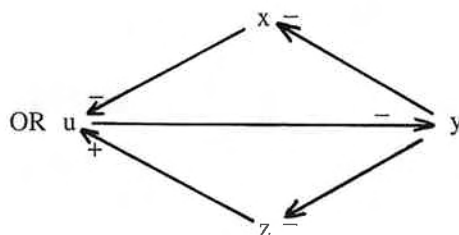
Until recently, it was believed that an asynchronous description (as used throughout this book) would be too complicated to be of practical use and that if it were nevertheless used, one could find "anything". We have pointed out³ that (1) it can be used practically, (2) the pattern of behavior is more complex than in the synchronous description, but nevertheless well defined (one does not find "anything"), and (3) as described at length in this book, what one finds bears a striking resemblance to the differential description.

In fact, one of the major differences between the synchronous and asynchronous descriptions is that in the former a positive loop can be trapped not only in one of its two stable states, but also in an oscillation made stable by the supposedly exact equality of the time delays.

In this appendix, we will use two systems. The first was presented in Chapter 4: it will be analyzed again to compare its behavior in synchronous and asynchronous descriptions. For the second system, we asked Kauffman to improvise a small "random" network for us and we analyze it using both the synchronous and the asynchronous treatment.

SIMPLE SYNCHRONOUS AND COMPLEX ASYNCHRONOUS BEHAVIOR OF A FOUR-ELEMENT SYSTEM

The system



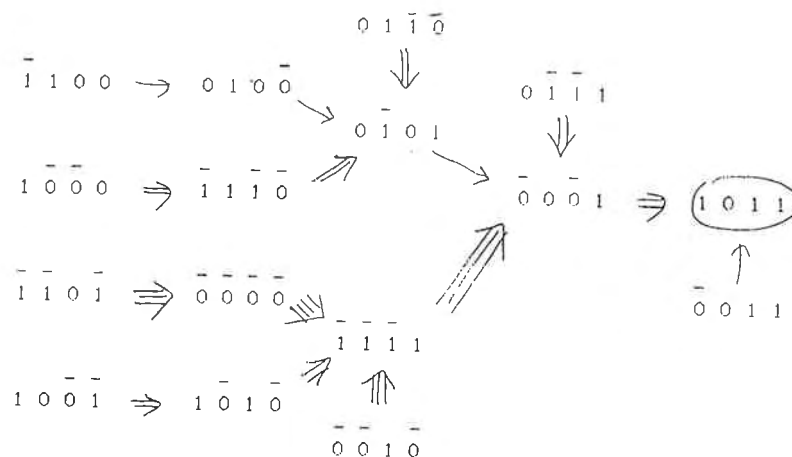


FIGURE 1. Synchronous graph of sequences of states for the System 1. Double, triple, and quadruple arrows refer to double, triple, and quadruple commutations, respectively.

with logical relations:

$$\begin{aligned}
 X &= \bar{y} \\
 Y &= \bar{u} \\
 Z &= \bar{y} \\
 U &= \bar{x} + z
 \end{aligned}
 \tag{1}$$

was used in Chapter 4 to present the stability analysis of cycles. The state table can be found in Chapter 4 (Section III). In Figure 1 of the present chapter is shown the graph of sequences of states of the *synchronous* description. This graph illustrates the above comments.

It can be seen that several states may converge onto one subsequent state, but no state diverges onto two subsequent states. In the present case, all trajectories lead to the unique stable state 1011.

In contrast to this simple behavior, we have already seen (Chapter 4) that the asynchronous system has a choice between two attractors, the stable state 1011 and a cyclic attractor that has several possible pathways (precise cyclic sequences of states) according to the values of the time delays. This situation was later found in the differential description of the system. For appropriate values of the parameters, the differential system has a choice between a stable steady state (stable node) and a limit cycle whose precise morphology depends on the parameter values. These domains are separated by a separatrix "hypersurface" (three-dimensional because the system itself has four variables) on which lies a third steady state, a saddle-focus, which is approached along the separatrix in a periodic way, then departed from in the direction of either attractor. Note that the logical analysis describes an unstable cycle, obviously the logical equivalent of this saddle focus.

In Figure 2 are shown the seven elementary forms (C^0 to $C7^0$) of the cyclic attractor. They have six, eight, or ten states, four of which (from $0\bar{1}01$ to $1\bar{0}00$) are common to all cycles. As one can guess from the fact that not all states have subscripts, and confirm by actual analysis, they are stable cycles. In other words, for each of them, there is a volume in the space of the time delays such that once the system has begun to follow this cycle, it will follow it indefinitely.

TABLE 1
The Network Used in This Paper,
as Described by A Set of Logical Equations

$$\begin{aligned}
 Y_1 &= y_6 \cdot y_{12} \\
 Y_2 &= \bar{y}_1 \cdot y_4 \\
 Y_3 &= \bar{y}_9 + y_4 \\
 Y_4 &= \bar{y}_4 + \bar{y}_7 \\
 Y_5 &= \bar{y}_{11} \cdot y_{15} \\
 Y_6 &= y_{13} + y_5 \\
 Y_7 &= \bar{y}_3 \cdot \bar{y}_6 \\
 Y_8 &= y_9 \cdot \bar{y}_8 \\
 Y_9 &= y_{12} + \bar{y}_{14} \\
 Y_{10} &= y_{14} + y_1 \\
 Y_{11} &= \bar{y}_1 \cdot y_6 \\
 Y_{12} &= \bar{y}_1 + y_9 \\
 Y_{13} &= y_3 \cdot \bar{y}_{10} \\
 Y_{14} &= \bar{y}_7 + \bar{y}_1 \\
 Y_{15} &= y_8 + y_3 \\
 Y_{16} &= y_2 \cdot \bar{y}_{15}
 \end{aligned}$$

The cycle labeled $C1^1$ is interesting because two states, $100\bar{1}$ and $1\bar{0}00$, are present twice in its sequence, with different subscripts. A closer examination shows that cycle $C1^1$ can be built by incorporating the sequence of the unstable cycle into the cycle $C1^0$. It is nevertheless a stable cycle, as shown by the fact that there are states without an index (and confirmed by analysis). In fact, from each of the "elementary" cycles $C1^0$ to $C7^0$, one can derive an unlimited number of complex cycles $C1^1 \dots C1^n \dots C2^1 \dots C2^n$, etc. by incorporating 1, 2, ..., n , ... times the states of the unstable cycle. These are all stable cycles, but their domain of stability in the space of time delays becomes thinner and thinner as n increases. When $n \rightarrow \infty$, we tend to the unstable cycle whose domain of stability is infinitely thin (a "hypersurface" in the space of the time delays). This situation is discussed in Chapter 4, Section III.

A 16-ELEMENT NETWORK PROPOSED BY KAUFFMAN

INTRODUCTION

The system analyzed here uses 16 variables (y_1 to y_{16}). Each Boolean function is a function of two of these variables, as indicated in Table 1. Which variables serve as input of a function, and how they interact, has been "chosen at random" by Kauffman. The system was wired up on the logical machine "Delphine" and successively treated in synchronous terms (equal time delays) and in asynchronous terms (unequal time delays).

SYNCHRONOUS TREATMENT

For the first simulations we used equal delays (synchronous treatment). After some time (see below) the system stabilized in a situation in which 10 of the 16 variable are locked in either the "on" (variables $y_3, y_4, y_9, y_{10}, y_{12}, y_{14}$, and y_{15}) or the "off" (variables y_7, y_{13}, y_{16}) position. The other six variables (y_1, y_2, y_5, y_6, y_8 , and y_{11}) oscillate permanently. The sequence of states from 000..., arbitrarily taken as the initial states, is given in Table 2.

TABLE 2
Synchronous Treatment: The Temporal Sequence of States of the Variables,
Taking 000... as the Initial State

State	y ₁	y ₂	y ₃	y ₄	y ₅	y ₆	y ₇	y ₈	y ₉	y ₁₀	y ₁₁	y ₁₂	y ₁₃	y ₁₄	y ₁₅	y ₁₆
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	1	1	0	0	1	0	1	0	0	1	0	1	0	0
3	0	1	1	0	0	0	0	1	1	1	0	1	1	1	1	0
4	0	0	0	1	1	1	0	0	1	1	0	1	0	1	1	0
5	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0	0
6	1	0	1	1	0	1	0	0	1	1	1	1	0	1	1	1
7	1	0	1	1	0	0	0	1	1	1	1	1	0	1	1	0
8	0	0	1	1	0	0	0	0	1	1	0	1	0	1	1	0
9	0	1	1	1	1	0	0	1	1	1	0	1	0	1	1	0
10	0	1	1	1	1	1	0	0	1	1	0	1	0	1	1	0
11	1	1	1	1	1	1	0	1	1	1	0	1	0	1	1	0
12	1	0	1	1	1	1	0	0	1	1	1	1	0	1	1	0
13	1	0	1	1	0	1	0	1	1	1	1	1	0	1	1	0
14	1	0	1	1	0	0	0	0	1	1	1	1	0	1	1	0
15	0	0	1	1	0	0	0	1	1	1	0	1	0	1	1	0
16	0	1	1	1	1	0	0	0	1	1	0	1	0	1	1	0
17	0	1	1	1	1	1	0	1	1	1	0	1	0	1	1	0
18	1	1	1	1	1	1	0	0	1	1	0	1	0	1	1	0
19	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0
20	1	0	1	1	0	1	0	0	1	1	1	1	0	1	1	0
Summary	-	-	1	1	-	-	0	-	1	1	-	1	0	1	1	0

After 7 steps, the system reaches a 14-step cycle. It has been checked that the cycle is still found after having let the machine run for a long time. Also, the same cycle is reached from 111... and various other states taken as initial states.

Note that these *synchronous* simulations can be performed easily on a pocked calculator, with the same result.

ASYNCHRONOUS TREATMENT

The same process has been repeated using different arbitrary values for the time delays (asynchronous treatment).

For a first set of time delays (see Table 3), the result was at first view surprisingly similar to that of the synchronous simulation. In fact, after some time, the same elements of the system were stabilized at the same logical values. The sequence of states was, of course, different, if only because the asynchronous character of the run renders simultaneous commutations of the variables infrequent.

But the interesting point is that instead of a well-defined cycle, one finds irregular periodicity, with a "period" close to the 14 found in the synchronous case. These first results would thus suggest that the behavior of the system is somehow buffered against variations

TABLE 3
The Time Delays Used in our First Asynchronous Simulation

	y_1	y_2	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}
ϵ	90	130	70	140	200	10	210	500	200	103	230	52	102	150	91	4
δ	110	80	110	50	190	40	95	105	110	300	100	15	70	30	7	400

Note: ϵ refers to the "on" delays, δ to the "off" delays.

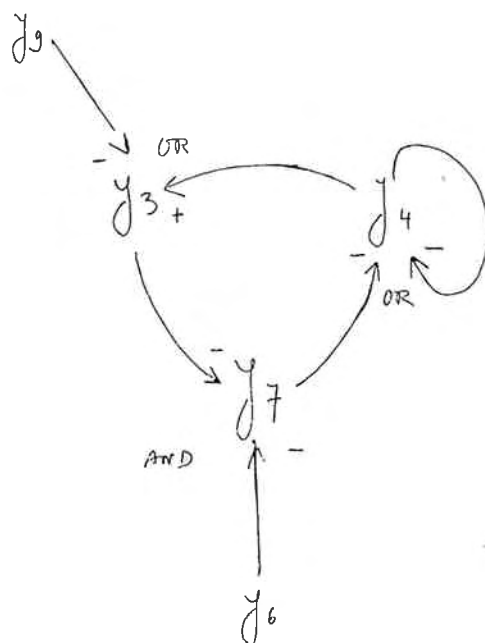


FIGURE 3. A subsystem comprising the loop $y_3 - y_4 - y_7$ and the variables directly connected to it.

of the time delays, in the sense that in spite of quite different values of the time delays, the general pattern remains very similar to the synchronous one. However, the aperiodic behavior is qualitatively interesting.

The structure of the network was then analyzed to identify the loops. It soon appeared that the positive loop

$$y_3 \xrightarrow{+} y_4 \xrightarrow{-} y_7 \xrightarrow{+} y_3$$

plays a central role in the network. If isolated, this loop would have a choice between two stable states, (110) and (001).

If, in addition to the loop $y_3 - y_4 - y_7$, one considers the elements directly connected to it, one gets the subsystem of Figure 3, in which variables y_6 and y_9 are treated as if they were external variables whose value can be imposed from outside. The equations are

$$Y_3 = y_4 + \bar{y}_9$$

TABLE 4
The Complete (a) and Compact (b) State Tables of the Subsystem
Described in Figure 3

(a)					
$y_3y_4y_7$	00	01	11	10	y_6, y_9
000	111	011	010	110	
001	111	011	010	110	
011	101	101	100	100	
010	111	111	110	110	
110	110	110	110	110	
111	100	100	100	100	
101	110	010	010	110	
100	110	010	010	110	
$y_3y_4y_7$					

(b)					
	00	01	11	10	y_6, y_9
$\bar{0}\bar{0}\bar{0}$	$\bar{0}\bar{0}\bar{0}$	$\bar{0}\bar{0}\bar{0}$	$\bar{0}\bar{0}\bar{0}$	$\bar{0}\bar{0}\bar{0}$	
$\bar{0}\bar{0}\bar{1}$	$\bar{0}\bar{0}\bar{1}$	$\bar{0}\bar{0}\bar{1}$	$\bar{0}\bar{0}\bar{1}$	$\bar{0}\bar{0}\bar{1}$	
$\bar{0}\bar{1}\bar{1}$	$\bar{0}\bar{1}\bar{1}$	$\bar{0}\bar{1}\bar{1}$	$\bar{0}\bar{1}\bar{1}$	$\bar{0}\bar{1}\bar{1}$	
$\bar{0}\bar{1}\bar{0}$	$\bar{0}\bar{1}\bar{0}$	$\bar{0}\bar{1}\bar{0}$	$\bar{0}\bar{1}\bar{0}$	$\bar{0}\bar{1}\bar{0}$	
$\textcircled{110}$	$\textcircled{110}$	$\textcircled{110}$	$\textcircled{110}$	$\textcircled{110}$	
$1\bar{1}\bar{1}$	$1\bar{1}\bar{1}$	$1\bar{1}\bar{1}$	$1\bar{1}\bar{1}$	$1\bar{1}\bar{1}$	
$1\bar{0}\bar{1}$	$1\bar{0}\bar{1}$	$1\bar{0}\bar{1}$	$1\bar{0}\bar{1}$	$1\bar{0}\bar{1}$	
$1\bar{0}\bar{0}$	$1\bar{0}\bar{0}$	$1\bar{0}\bar{0}$	$1\bar{0}\bar{0}$	$1\bar{0}\bar{0}$	
$y_3y_4y_7$	$y_3y_4y_7$	$y_3y_4y_7$	$y_3y_4y_7$	$y_3y_4y_7$	

Note: The variables in the positive loop ($y_3 - y_4 - y_7$) are treated as internal variables (lines). Variables y_6 and y_9 are treated as input variables (columns).

$$Y_4 = \bar{y}_4 + \bar{y}_7$$

$$Y_7 = \bar{y}_3 \cdot \bar{y}_6$$

In Table 4, we find the behavior of this subsystem.

We know that the stable state $\textcircled{110}$ exists in the *complete* network, since as in all cases considered so far, the elements y_3 , y_4 , and y_7 are locked in states 1, 1, and 0, respectively. If

the loop is in the state (110), then a number of elements have their value fixed:

$$y_7 = 0 \text{ imposes } y_{14} = 1$$

$$y_{14} = 1 \text{ imposes } y_{10} = 1$$

$$y_{10} = 1 \text{ imposes } y_{13} = 0$$

$$y_3 = 1 \text{ imposes } y_{15} = 1$$

$$y_{15} = 1 \text{ imposes } y_{16} = 0$$

We show this partial analysis because it accounts for a large part of the situations met to this point: when the loop y_3 — y_4 — y_7 is in its state (110), y_3 , y_4 , y_{10} , y_{14} , and y_{15} are, and y_9 and y_{12} may be, locked at value 1, while y_7 , y_{13} , and y_{16} are locked at 0.

Since up to now, one of these states ((110)) of the positive loop y_3 — y_4 — y_7 accounts for the state, on or off, of most stabilized elements, it was reasoned that an efficient way to modify the behavior of the whole system might consist in finding time delays that would not lock the loop in this position. When such a set of time delays is used, the picture becomes completely different, indeed. For example, if we set $\epsilon_{y_3} = 370$ instead of 70, we get:

$$0 \quad - \quad - \quad - \quad 0 \quad 0 \quad - \quad - \quad 1 \quad 1 \quad 0 \quad 1 \quad 0 \quad 1 \quad - \quad 0$$

and if, in addition, we set $\delta_{y_4} = 250$, instead of 50, we get:

$$0 \quad 0 \quad 0 \quad - \quad 0 \quad 0 \quad 1 \quad - \quad 1 \quad 1 \quad 0 \quad 1 \quad 0 \quad 1 \quad - \quad 0$$

as compared to the situation:

$$- \quad - \quad 1 \quad 1 \quad - \quad - \quad 0 \quad - \quad 1 \quad 1 \quad - \quad 1 \quad 0 \quad 1 \quad 1 \quad 0$$

obtained with the original set of time delays and also in the synchronous treatment.

Finally, one may ask whether the irregular behavior mentioned above is chaos or simply some kind of multiple periodicity. In the case analyzed here, it can be shown to be due simply to the coexistence of two negative loops with noncommensurable periods. This is not true chaos.

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